"EVALUATING THE EFFECTIVENESS OF TOPICAL APPLICATION OF PURE NATURAL HONEY AND BENZYDAMINE HYDROCHLORIDE ON RADIATION - INDUCED MUCOSITIS"

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Certificate

Certified that the dissertation on "**EVALUATING THE EFFECTIVENESS OF TOPICAL APPLICATION OF PURE NATURAL HONEY AND BENZYDAMINE HYDROCHLORIDE ON RADIATION - INDUCED MUCOSITIS"** done by **Dr. N. BALAJI,** Post Graduate student (M.D.S.), Branch VII Oral Medicine and Radiology, Tamilnadu Government Dental College and Hospital, Chennai submitted to The Tamilnadu Dr. M.G.R. Medical University for partial fulfilment of the M.D.S. Degree Examination in April 2011, is a bonafide research work done under my guidance and supervision.

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CONTENT

INTRODUCTION

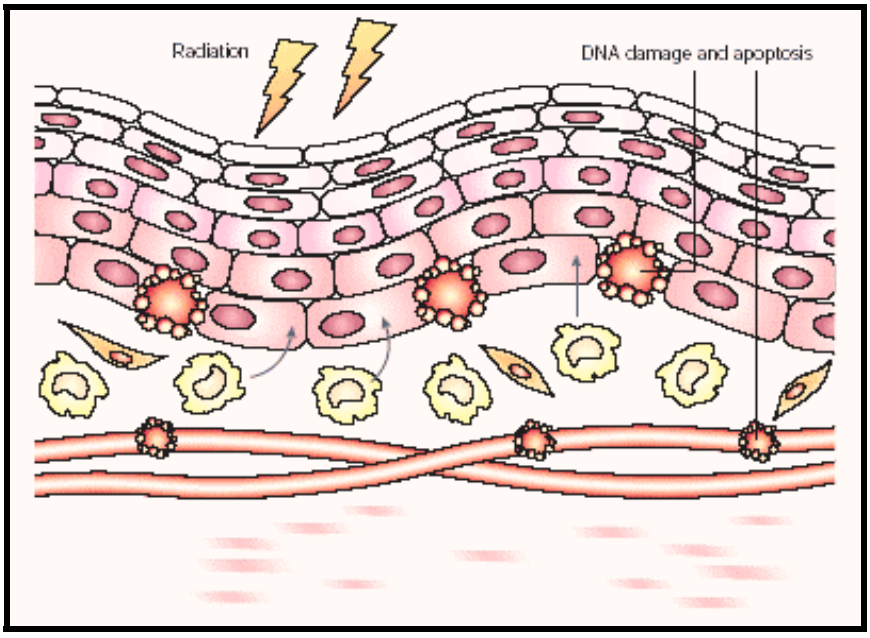
Radiotherapy, also called radiation therapy, is the treatment of cancer and other diseases with ionizing radiation. Ionizing radiation deposits energy that injures or destroys cells in the area being treated (the "target tissue") by damaging their genetic material, making it impossible for these cells to continue to grow. Although radiation damages both cancer cells and normal cells, the latter are able to repair themselves and function properly. Radiotherapy is indicated for majority of head and neck cancer patients. Depending on the extent and stage of the malignancy it can be either used alone or in combination with chemotherapy or surgery.

Mucositis is the painful inflammation and ulceration of the mucous membranes lining the digestive tract, usually as an adverse effect of chemotherapy and radiotherapy treatment for cancer[61]. Mucositis can occur anywhere along the gastrointestinal (GI) tract, but oral mucositis refers to the particular inflammation and ulceration that occurs in the mouth. Oral mucositis is a common and often debilitating complication of cancer treatment.[66]

Floyd had defined oral mucositis as the inflammatory change of the oral mucosa resulting from the direct effect of radiotherapy. [26]

According to Beumer et al, inhibition of cell growth and maturation by radiation disrupts the primary mucosal barrier of the mouth and throat and thereby creates a pathway for the establishment of oropharyngeal infection by resident oral microflora. The consequences of this include oral mucositis and gingivitis, oral candidiasis, xerostomia, trismus, dental caries, osteoradionecrosis, cellulitis, and viral mucosal eruptions.[2]

Radiation Induced Damage To The Oral Mucosa



The first clinical signs of radiation-induced mucositis occur at the end of the first week of a conventional seven-week radiation protocol (daily dose of 1.8 to 2.0 Gy, five times a

week). A white discoloration of the oral mucosa, which is an expression of hyperkeratinisation of the epithelium, followed by erythema, is initially seen. In other cases, a white discoloration maybe observed in combination with areas of erythema or erythema may appear first. The above clinical signs represent the grade I mucositis[86] and are mostly asymptomatic. Towards the end of the second or around the third week of radiotherapy, small foci of ulceration can be observed, corresponding to the grade II mucositis. Patient complains of mild pain and can take soft diet. Severe, grade III mucositis presents as ulcers covered by pseudomembranes, affecting large areas of the oral mucosa . Grade IV mucositis represents even more severe ulceration, covering almost all mucosal surfaces. Patient complains of severe pain, can take liquids only or may necessitate nasogastric tube or parenteral support.

Mucositis generally persists throughout radiotherapy, and develops at its maximum grade at the end of the irradiation period. One to three weeks or more, depending on the severity, are needed for mucositis to heal, after the completion of radiotherapy.

Erythematous, ulcerated and xerostomic (dry) oral mucosa serves as site for the development of secondary infection. During a course of curative radiation, about 80% of the patients

will develop different grades of mucositis. Mucositis may be so severe as to delay treatment and so limit the effectiveness of cancer therapy.[5]

The extent of the injury is directly related to the mucosal volume irradiated, anatomic subsite exposed, treatment intensity, and individual patient predisposition . In radiotherapy mucositis is an integral part in terms of morbidity, as during a course of curative radiation the majority of patients will develop pseudomembranous mucositis. OM from radiotherapy peaks at about weeks 5 to 6 and typically resolves during weeks 8 to 12 of follow-up.[25]

The early radiation reaction causes local discomfort as well as difficulties in drinking, eating, swallowing and speech. In head and neck radiotherapy these more aggressive regimens have been shown to improve local tumor control, but these are related to an increase in severe mucositis. This higher rates of acute toxicity result in higher levels of pain and difficulty in oral intake, and a significant worsening of the patient's quality of life. Recent data have shown that more than half of the head and neck cancer patients (56%) who receive altered fractionation radiotherapy, will experience more severe mucositis as compared to 34% of patients who receive conventional radiotherapy[3].

Radiation damage is anatomically site-specific; toxicity is localized to irradiated tissue volumes. Degree of damage is dependent on treatment regimen-related factors including type of radiation used, total dose administered, and field size/fractionation. Head and neck radiation can also induce damage that results in permanent dysfunction of vasculature, connective tissue, salivary glands, muscle, and bone. Loss of bone vitality occurs secondary to both injury to osteocytes, osteoblasts, and osteoclasts as well as from a relative hypoxia due to reduction in vascular supply. These changes can lead to soft tissue necrosis and osteonecrosis that result in bone exposure, secondary infection, and severe pain.[49]

Severe mucositis can give rise to nutritional problems, while hospitalisation and nasogastric feeding may become necessary. Rates of hospitalisation due to severe mucositis, reported in several studies, were 32% for altered fractionation radiotherapy and overall 16% of all types of radiotherapy. Furthermore, about 10% and up to 30% of patients, depending mostly on the type of treatment, may necessitate an interruption or a modification and prolongation of the course of sradiotherapy because of severe mucositis [59,67]. Interruptions and prolonged treatment adversely affect outcome and therapeutic effect**.** While oral complications primarily are

associated with discomfort and interference with oral function, in patients who are also immunocompromised or debilitated, these complications can become life threatening[50].

The Consensus Development Panel of the National Institutes of Health (Consensus statement, 1990) [52] stated that no drugs can prevent mucositis, an opinion that still holds to date.

Consequently, treatment of mucositis is still limited to reduction of its severity. Oral care programs, relief of pain and discomfort, early diagnosis and treatment of concomitant secondary mucosal infections and/or strategies to eliminate micro-organisms, that are thought to promote or aggravate mucositis, are all engaged in its treatment. MASCC [48] and NCCN [16] guidelines and a National Cancer Institute report recommend “basic oral care” as a standard practice to prevent infections and potentially help alleviate mucosal symptoms.[31,68]

The maintenance of oral hygiene during and after radiation will reduce the risk for dental complications, including infections, caries, gingivitis, and osteoradionecrosis. Basic oral care during radiation involves brushing in a nontraumatic fashion with a soft brush, flossing as tolerated, and frequent rinsing with bland solutions such as normal saline

with sodium bicarbonate (1 L water with 1/2 teaspoon baking soda and 1/2 teaspoon salt), the use of moisturizing agents, periodic dental evaluations and cleanings, and the use of lifelong daily dental fluoride prophylaxis [24]

Many different treatments are used to prevent or treat mucositis. General oral care protocols are to be followed. Mouthwashes with mixed action immunomodulatory agents, topical anesthetics, antiseptics, antibacterial, antifungal and antiviral drugs are used. Mucosal barriers, coating agents, cytoprotectants, mucosal cell stimulants, psychotherapy are also used in the management protocol.

Many agents of differing mechanisms of action have been used in the prevention and treatment of oral mucositis induced by anticancer therapies. Currently, no intervention is completely successful at preventing or treating oral mucositis. The several solutions, drugs and methods used and studied in the prophylaxis and therapy of chemotherapy or radiotherapy-induced oral mucositis reflects the need of new, more efficient tools in the management of this complication [14]

AIMS AND OBJECTIVES

1. To evaluate the effectiveness of pure natural honey on onset and severity of radiation - induced mucositis.

2. To evaluate the effectiveness of benzydamine hydrochloride on onset and severity of radiation - induced mucositis.

3. To compare the effectiveness of pure natural honey and benzydamine hydrochloride on onset and severity of radiation – induced mucositis with control.

REVIEW OF LITERATURE

Head and neck irradiation can cause a wide spectrum of oral complications. Ulcerative oral mucositis is a virtually universal toxicity resulting from this treatment; there are clinically significant similarities as well as differences compared with oral mucositis caused by chemotherapy. [58, 69, 70] Head and neck radiation can also induce damage that results in permanent dysfunction of vasculature, connective tissue, salivary glands, muscle, and bone. Loss of bone vitality occurs secondary to both injury to osteocytes, osteoblasts, and osteoclasts as well as from a relative hypoxia due to reduction in vascular supply. These changes can lead to soft tissue necrosis and osteonecrosis that result in bone exposure, secondary infection, and severe pain. [49]

Effect of radiation on oral and salivary gland tissues:

Radiotherapy for head and neck tumours is a viable treatment modality. Radiotherapy is concerned with the delivery of the correct radiation dose to the tumour mass while minimizing the dose received outside the tumour zone. Rothwell states that most orofacial complications are dose dependent and that severe side effects occur when doses greater than 45 Gy are administered bilaterally to the mouth,jaws and salivary glands.

Teeth:

Irradiation of teeth with therapeutic doses during their development severely retards their growth. Children receiving radiation therapy to the jaws may show defects in the permanent dentitions such as retained root development, dwarfed teeth or failure to form one or more teeth.[13,27,71]

Radiation caries is a rampant form of dental decay that may occur in individuals who receive a course of radiotherapy that includes exposure to salivary glands. The carious lesions result from changes in the salivary glands and saliva, including reduced flow, decreased pH, reduced buffering capacity and increased viscosity.Irradiation of teeth by itself does not influence the course of radiation caries[87].

Bone:

The primary damage to mature bone results from radiation induced damage to the vasculature of the periosteum and cortical bone. Radiation also acts destroying osteoblasts and to a lesser extent, osteoclasts..Subsequent to radiation, normal marrow may be replaced with fatty marrow and fibrous connective tissue. The marrow tissue becomes hypovascular, hypoxic and hypocellular. When these changes are so severe, that sbone death results, the condition is termed Osteoradionecrosis [23,47,54]

Effect of radiation on salivary glands:

Ionising radiation causes glandular tissue damage, which may result in a rapid, irreversible loss of salivary fluid secretion. The glandular architecture is replaced by ductal remnants and loose fibrous connective tissue which is moderately infiltrated with lymphocytes and plasma cells. This progressive glandular, atrophy, fibrosis and reduced salivary output begins slowly after initial exposure and intensifies there after [50].

Taste buds:

Taste buds are sensitive to radiation. Patients often note loss of taste acuity during the 2nd and 3rd week of radiotherapy, which may proceed to a state of virtual in sensitivity, with recovery to near normal level, some 60 to 120 days after irradiation[54,87]

The severity of oral mucositis can range from barely perceptible mucosal erythema and atrophy to severe mucosal inflammation and ulceration. Oral mucositis can obviously cause severe pain and add significantly to the morbidity of cancer therapy. Oral tissue damage and pain can result from a number of different processes related not only to the cancer therapy but also to a number of patient-related factors as well [72]

Mucosal Characteristics

The primary function of oral mucosa is to serve as a barrier that protects the underlying tissues and organs [73]. The tissues of the oral cavity are continually subjected to a wide variety of traumatic insults associated with oral function and are under constant pressure from a wide variety of microflora inhabiting the oral cavity. Key to withstanding these insults is the ability of the basal cells in the deeper germinative layers of the oral epithelium to continually divide and follow an orderly process of maturation and migration, culminating in desquamation at the surface. Comparatively, the turnover rate (time from basal replication to desquamation) for the oral mucosa is less than that of the gut epithelium but greater than that of skin. The epithelial renewal rate for oral mucosa varies with different anatomical areas of the mouth with the nonkeratinized lining mucosa (e.g., ventral tongue, labial mucosa, soft palate) having rates that have been estimated to be 1.5 to five times greater than masticatory mucosa (e.g., attached gingiva, hard palate) [62]. The epithelial cells of the oral mucosa undergo rapid turnover, usually every 7 to 14 days,which makes these cells sustain effects of cytotoxic therapy[50]. Radiation therapy and chemotherapy primarily

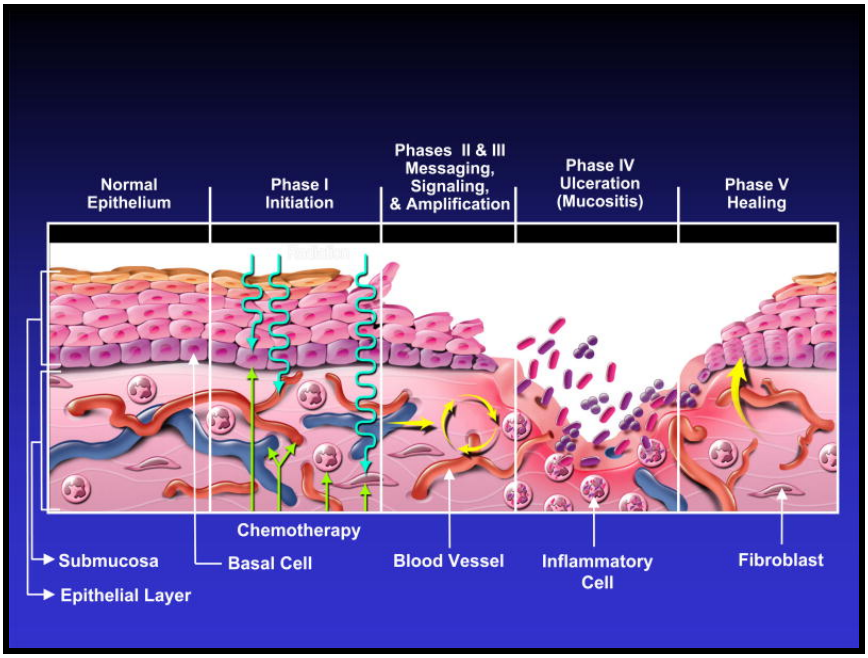
damage and kill dividing cells by damaging DNA or interfering with cell division.

Saliva serves a broad spectrum of physiologic needs relative to oral health and function[45,46]. The functions of saliva include (a) lubrication of oral tissues (and food) to prevent physical trauma and irritation, (b) hydration of mucosal surfaces, and (c) modulation and control of microbial colonization and growth due to the presence of salivary proteins, antibodies, and other proteins with antimicrobial actions.. Consequently, salivary gland dysfunction will not only result in complaints of a ``dry mouth'' but will also increase the risk of trauma and irritation to oral tissues and increase the risk of oral mucosal infections[74]

In addition to the damage to the mucosal epithelium, radiation causes damage and subsequent changes in the submucosal connective tissue and vasculature, even though the cellular proliferation rate and turnover in these tissues is much lower[44]. Damage to the connective tissue component of the oral mucosa is noted as hyalinization of collagen, depolymerization of large molecules making up the ground substance, increased vascular permeability, tissue edema, and presence of an inflammatory infiltrate [6,66]. Radiation to the endothelial cells of the small vessels and capillaries results in vasculature damage

that reduces functional efficiency of the vessels. Osseous damage from radiation results in: marrow avascularity and fatty degeneration; reduction of osteocytes, osteoblasts, and osteoclasts; and microfractures and enlarged lacunae [78]. Many of the chronic complications of radiation therapy result from nonrepairable damage to the vasculature, connective tissue, and bone cells that leads to hypovascularity, tissue ischemia, fibrosis, and nonvital bone[44].

Biological stages of mucositis



The five biological stages of mucositis can be defined as initiation, primary damage response, signal amplification, ulceration and healing.

Initiation

The initiation stage of tissue injury occurs rapidly following the administration of radiation or chemotherapy. Radiation and chemotherapy initiate both DNA and non-DNA damage. DNA strand breaks result in direct cellular injury that targets cells in the basal epithelium as well as cells within the submucosa. Simultaneously, reactive oxygen species (ROS), which are crucial mediators of downstream biological events, are generated. Although the mucosa seems to be absolutely normal at this stage, a cascade of events begins in the submucosa that ultimately results in mucosal destruction. Denham and Hauer-Jensen reported that although there is death of some cells within the basal and suprabasal epithelium, it is the destruction of the cells in the underlying submucosa that makes the largest contribution to injury[17].

Primary Damage Response

DNA damage, non-DNA damage and ROS initiate an interesting and complex series of events that is still being defined. DNA strand breaks result in the activation of several transduction pathways that activate transcription factors such as p53 and nuclear factor-κB (NF-κB)[11] NF-κB is activated in response to radiation, several chemotherapeutic drugs and ROS(Cell-membrane-bound molecules that are released during lipid

peroxidation result in the upregulation of immediate-response genes, such as those encoding c-JUN and and c-JUN amino-terminal kinase (JNK)[18] These upregulate other transcription factors, such as NRF2 (REF. 36). Importantly, all of these changes occur in all of the cells and tissues that comprise the mucosa — not just in the epithelium.

Among the transcription factors that are activated by radiation, chemotherapy and ROS, NF-κB has been suggested to be one of the most significant, in relation to both toxicity and resistance of tumours to therapy. [39,10]

Signal Amplification

As a consequence of the gene upregulation that is brought about by the initial activation of transcription factors, a broad range of biologically active proteins accumulate and target the tissues of the submucosa. Some of these, particularly the pro-inflammatory cytokines, not only damage tissue, but also provide a positive-feedback loop to amplify the primary damage that is initiated by radiation or chemotherapy. This pathway ultimately results in the activation of caspase 3 and in cell death[18]**.** Importantly, although the mucosal cellular targets include the epithelium, they also include elements of the submucosa. TNF-α also activates sphingomyelinase. So, its increased level in the tissue amplifies pro-apoptotic signals that

are mediated by the ceramide pathway. In addition, both TNF-α and IL-1β induce MMP1 and MMP3 activation.[7,82]. It is noteworthy that while the ravages of biological chaos are occurring during the initial phases of mucositis, the clinical picture is one of quiescence. Although there might be some mucosal erythema during these stages, for the most part, tissue integrity is in place and patients have few symptoms. This changes as ulceration develops.

Ulceration

The ulcerative phase of mucositis is the most significant to both the patient and the caregiver. The loss of mucosal integrity results in extremely painful lesions that are prone to superficial bacterial colonization. In the case of neutropenic patients, these breaks in the mucosa serve as portals of entry for the numerous microorganisms that reside in the mouth, and often lead to bacteraemia and sepsis. In addition, cell-wall products from colonizing bacteria are likely to penetrate into the submucosa, where they activate infiltrating mononuclear cells to produce and release additional pro-inflammatory cytokines[19]. This probably promotes the expression of pro-apoptotic genes[1] and potentiates tissue injury. Inflammatory cells then migrate by chemotaxis to the base of the lesion, where they produce damaging enzymes.

Healing

In most cases, mucositis is an acute phenomenon that is self-resolving once cancer therapy ends. Although there are parallels between the resolution of mucositis-induced ulcers and the healing of other types of mucosal injury, it is likely that the sequence of events that leads up to mucositis modulates the process. Signals from the submucosal extracellular matrix and mesenchyme govern the rate of epithelial-cell migration, the rate of proliferation and the differentiation of healing tissue. The course of tissue behaviour must be regulated to some degree by the type of cancer treatment (radiation versus chemotherapy), the agents selected and the dose and timing of therapy.

In addition to mucosal damage, ionizing radiation can cause significant salivary gland dysfunction, although the exact mechanism of the damage is not clear [32,45,46,73]. Damage does not appear to be entirely due to direct acinar damage and there is some evidence suggesting that damage to ductal epithelium and vasculature may be responsible for the decreased salivary flow rates. Damage to the major and minor salivary glands by radiation also reduces levels of salivary antibodies and other antimicrobial proteins and glycoproteins, which contributes to increased mucosal irritation and infection. Additionally, saliva

becomes more acidic and more mucinous in character [45,46,62,73].

Different agents have been used for the prophylaxis as well as management of Radiation-induced mucositis. Pain management is the single most important aspect of symptom control during HN radiation [20] The agents recommended or tested for the prevention and management of radiation induced mucositis, have targeted specific pathways and they include mucosal coating agents, anti-inflammatory agents, antimicrobials, immunomodulators, anesthetics and analgesics, and other agents that are difficult to classify.

These locally applied as well as systemically taken agents have been supportive at most, consisting of measures to alleviate pain and improve discomfort, support adequate hydration and, in some, the ability to eliminate secondary infections [88].

B. M. Biswal et al 2001 studied the effect of topical application of pure natural honey on radiation- induced oral mucositis. There was significant reduction in the symptomatic grade 3 and 4 mucositis among honey-treated patients compared to controls; i.e. 20% versus 75% (p 0.00058).The compliance of honey-treated group of patients was better than controls. The difference in grade3 and 4 mucositis was 20% and 75%

respectively in the treatment and control arm ($p < 0.00058$).Thus in this study it was seen that although there was no significant change in grade 1 & 2 mucositis, grade3 & 4 mucositis was significantly reduced in the treatment arm.[4]

Motallebnejad et al in 2004 studied the effect of topical application of pure natural honey on radiation mucositis. In this randomized single blind clinical trial with 40 patients, it was observed that the mucositis score of OMAS(Oral Mucositis Assessment Scale)[76] at the end of each week in the study group was significantly lower than the control group ($p=0.000$) thus showed that honey had a remarkable effect on radiation mucositis. [43]

Rashad U M et al in 2006 studied the efficacy of pure natural honey as prophylaxis against radiochemotherapy-induced mucositis. In this clinical trial of 40 patients, it was observed that only 15% of patients (3 patients) developed grade 3 mucositis, none in treatment group developed grade 4 mucositis. In the control group, 13 patients developed grade 3 or 4 mucositis ($p < 0.05$).

This study shows that prophylactic use of pure natural honey was effective in reducing mucositis resulting from radiochemotherapy in patients with head and neck cancer [63].

Khanal et al in 2010 in a single-blinded, randomized, controlled clinical trial, comparing natural honey and lignocaine, observed that only one in honey group developed intolerable oral mucositis compared with lignocaine group, indicating that honey is strongly protective against the development of mucositis. The proportion of patients with intolerable oral mucositis was lower in the honey group and this was statistically significant (p= 0.000).[32]

Joel B Epstein et al in 2001 evaluated the effect of 0.15% benzydamine hydrochloride on Radiation- induced mucositis. In this study it was observed that during conventional RT,regimens upto cumulative does of 5000 cGy, benzydamine (n= 69) significantly (p= 0.006) reduced erythema and ulceration by approximately 30% compared with the placebo (n= 76); greater than 335 of benzydamine subjects remained ulcer free compared with 18% of placebo subjects (p= 0.037%), and benzydamine significantly delayed the use of systemic analgesics compared with placebo (p< 0.05). Benzydamine was not effective in subjects (n= 20) receiving accelerated RT doses (>- 220cGy/day). [22]

In a double-blind/ placebo study was undertaken in order to ascertain the possible oral histoprotective effect of benzydamine mouthwash during the intra-arterial

polychemotherapy and/or radiotherapy for neoplasms of the head and neck. Statistical comparison of the results obtained in these two groups, treated with benzydamine and placebo mouthwash, revealed a marked difference in the clinical evolution of iatrogenic tissue damage. The benzydamine topical application was more effective than placebo in the control of mucositis symptoms and signs produced by radiotherapy and / or intra arterial antineoplastic chemotherapy.[29]

Kazemian A et al in 2005 studied the effect of benzydamine oral rinse on radiation mucositis and the results showed that the frequency of mucositis grade> or = 3, was 43.6% in contrast to 78.6% in other group (p= 0.001).Grade > or = 3 mucositis was 2.6 times more frequent in the placebo group. Intensity of mucositis increased upto 4^{th} week of treatment in both groups to grade 2.In the treated group the grade of mucositis was approximately constant to the end of therapy; but in the control group it raised to grade 3(p< 0.01).In this study it was inferred that benzydamine oral rinse seems to be effective, safe and well tolerated for prophylactic treatment of RIM in H& N cancers [33].

Kim JH et al (American journal of Clinical Oncology. 1986 apr;9(2):132-4.) in a double-blind, randomized clinical investigation studied the effect of benzydamine in radiation

induced mucositis. patients developed radiation mucositis,hyperemia,and throat pain when the total radiation dose reached above 2,000 rad over 2 weeks .Analysis of the data showed that benzydamine Hcl used as a rinse/gargle provided a statistically significant and clinically meaningful alleviation of the symptoms of oropharyngeal mucositis .[34]

Kamian et al in 2007 studied the effect of benzydamine hydrochloride on radiation mucositis in a randomized placebo controlled clinical trial. In this study it was observed that frequency of mucositis of grade 3 or more was found in placebo group compared to benzydamine group. The difference was statistically significant (p=0.001) oral rinse benzydamine was effective, safe, and well tolerated for prophylactic treatment of radiation-induced oral mucositis in head and neck tumors. [35]

King- fong cheng et al in 2006 in a prospective, randomized, and double blinded Studys, compared the efficacy of 0.2% chlorhexidine gluconate and 0.15% benzydamine hydrochloride oral rinses in alleviating irradiation mucositis for patients with head and neck cancer. The subjects were evaluated based on World Health Organization mucositis scale and the 10-cm visual analogue scale for mouth pain and dysphagia from this study, it was inferred that there was lessening of severity of mucositis, pain, and dysphagia for

patients with head and neck cancer receiving benzydamine oral rinse[36].

Samaranayake et al 1986 studied the the effect of benzydamine and chlorhexidine mouthwashes on radiation induced mucositis. Its efficacy as a mouthwash was compared with chlorhexidine in two groups of patients receiving radiotherapy for oral carcinoma. In this study it was noted that there was little difference between the two mouthwashes both in controlling pain and mucositis or in the oral carriage of the micro-organisms studied. [77]

Symonds R P et al in 1996 evaluated the effect of antibiotic pastilles on radiation mucositis, pain, dysphagia and weight loss in patients undergoing radical radiotherapy for head and neck cancer, in which it was observed that there was a considerable reduction in mucositis distribution, dysphagia, weight loss in the patients using antibiotic pastilles, indicating that the active pastilles had a beneficial effect on radiation induced oral mucositis.[78]

N. Kantardzic, V. Smajlbegovic, N. Kazic & A. Cardzic in 2008 evaluated the effectiveness of gelclair oral gel in the treatment of oral mucositis in patients with head and neck tumours during radiotherapy or/and chemotherapy. It was inferred from this study that there was a significant

improvement in management of pain and significant improvement in food intake in the study group.[55]

Cristina bez et al in 2008 evaluated the effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) mouthrinses as a potential treatment in reducing the duration of severe oral mucositis in patients undergoing bone marrow transplantation for hematologic malignancies. It was seen in this study that 60% of the GM-CSF mouthrinse patients had severe mucositis for less than 9 days, whereas only 28% of the controls had severe mucositis for less than 9 days. In addition, 10% of the GM-CSF mouthrinse patients experienced severe mucositis lasting 20 or more days, whereas 34% of the controls experienced severe mucositis for 20 or more days. It was inferred in this study that GM-CSF used as a rinse reduced the duration as well as severity of radition – induced mucositis.[12]

McAleese J J et al in 2000 assessed the effect of subcutaneous GM-CSF injections on acute radiation morbidity in patients undergoing accelerated radiotherapy for laryngeal cancer.In this prospective, randomized, observer-blind phase II trial, it was seen that mean duration of severe mucositis was less in GM-CSF group compared to the control group [51].

Kannan et al in 1997 in a pilot study evaluated the effect of GM-CSF mouth rinse on radiation induced mucositis. The

results showed that GM-CSF mouthrinse administration concurrently with conventional fractionated radiotherapy was feasible without significant toxicity. The acute side effects of radiotherapy namely mucositis, pain, and functional impairment were nil to minimal. The results are suggestive of mucosal protection by GM-CSF during radiotherapy.[37]

Meredith et al in 1997 in a double blind study, studied whether the addition of the ulcer-coating polysaccharide, sucralfate could improve symptomatic relief of radiation mucositis over a popular combination of antacid, diphenhydramine, and viscous lidocaine alone. There was statistically significant reduction in symptomatic radiation mucositis in the sucralfate group [65].

Etiz D et al in 2000 evaluated the effect of sucralfate on prevention of radition induced mucositis. In this prospective, randomized, clinical trial, it was inferred that sucralfate may be used as an preventive agent in the management of radiation induced mucositis. [21]

PD Madan Kumar, PS Sequeira, Kamalaksha Shenoy, Jayaram Shetty 2005 in a randomized clinical trial evaluated the effect of of three mouthwashes (0.12% chlorhexidine, 1% povidone-iodine, or salt/soda) on radiation-induced oral mucositis in patients with head and neck malignancies. The

results showed that patients in the povidone-iodine group had significantly lower mucositis scores when compared to the control group from the first week of radiotherapy. Oral mucositis due to antineoplastic radiotherapy.[42]

David M Brizel et al in 2000 in a randomized clinical trial, evaluated the effect of Amifostine as a radioprotector in patients undergoing radiotherapy. Amifostine was administered before irradiation. Amifostine reduced grade ≥ 2 acute xerostomia from 78% to 51% ($P < .0001$) and chronic xerostomia grade ≥ 2 from 57% to 34% ($P = .002$). Median saliva production was greater with amifostine (0.26 g *v* 0.10 g, $P = .04$). Amifostine did not reduce mucositis[15]

Dosia Antonadou et al in 2002 studied the prophylactic properties of amifostine against acute and late toxicities from radiochemotherapy in patients with head-and-neck cancer. In this randomized clinical trial, it was observed that amifostine reduced the grade IV mucositis and xerostomia.[16]

Kouvaris J et al studied the cytoprotective impact of amifostine against acute radiation mucositis. A significantly reduced severity of symptomatology related to oral, esophageal mucosa was noted in the amifostine group ($p < 0.05$).[38]

Ricardo Spielberger et al in 2004 in a double blind study, evaluated the effect of Palifermin with that of placebo on

mucositis in patients with hematologic malignancies. The mean duration of mucositis was 6 days in palifermin group. Compared to 9 days in control group 63% of palifermin group had severe mucositis(Grade 3 or 4 (WHO Mucositis Grading) compared to 98% in placebo group.[64]

Johannes et al in 1994 studied the effect of topical application of antibiotic pastilles in radiation induced mucositis Lozenges containing polymyxin E, tobramycin, and amphotericin B (PTA-lozenges) were polymyxin E, tobramycin, and amphotericin B may reduce irradiation mucositis of the oral cavity.[28]

Fred K. L. Spijkervet et al studied the effect of lozenges containing Polymyxin E, tobramycin, amphotericin B on radiation induced mucositis In this study it was observed that mucositis was significantly reduced in the study group[20].

Somkit Penpattanagul in 2007 evaluated the role of WF10-immunotherapy in reducing oro-pharyngeal complications in head and neck cancer chemoradiotherapy. Patients in the study that WF10 reduces the incidence of oro-pharyngeal complications, (including oral mucositis, dysphagia, oral pain), taste alteration and weight loss. The statistical significances were achieved for the parameters of oral mucositis ($p = 0.048$) and dysphagia ($p = 0.009$**).** [80]

MATERIALS AND METHODS

This study was conducted in The Department of Oral Medicine and Radiology, Tamilnadu Govt. Dental College & Hospital, Chennai-600003, and The Department of Radiation Oncology, Government General Hospital,Chennai-600003 from april 2010 to December 2010 after getting approved by the Institutional Ethical committee. The sample size comprised of 60 patients, of both genders within age groups of 30 years to 70 years, diagnosed with oral malignancy clinically and histopathologically. Of the sixty patients, the group comprised of 42 males and 18 females within ages of 30 to 70. The minimum age of female was 30 years of age and the maximum age was 65 years. The minimum age of male was 34 and the maximum age was 70 years (**Table 7).**

The patients were assigned into three groups by random sampling. Each group consisted of 20 patients. Group1 comprised of 11 (55%) males and 9 (45%) females. Group 2 comprised of 15 (75%) males and 5 (25%) females. Group 3(Control) comprised of 15 (75%) males and 5 (25%) females. In the group 1,the location of malignancies(Ca) were: Floor of mouth-1 patient, buccal mucosa -6 patients,Tongue-4 patients, Upper alveolus-3 patients, Lower alveolus-2patients,softpalate-3 patients, maxillary antrum-1patient(**Table 4**).The TNM

staging was done for group 1.The number of patients with TNM staging were: Stage I:No patient, Stage II:1 patient, Stage III: 1 patient, Stage IVa:16 patients, Stage IVb: 2 patients(**Table 5).**

In the group 2, the location of malignancies (Ca) were: Floor of mouth-no patient, buccal mucosa-5patients,Tongue-3patients Upper alveolus-5 patients, lower alveolus-4 patients,softpalate-2 patients, maxillary antrum-1 patient (**Table 4).** The TNM staging was done for group 2.The number of patients with TNM staging were: Stage I:No patient, Stage II:1 patient, Stage III: No patient, Stage IVa:16 patients, Stage IVb: 2 patients(**Table 5).**

In the group 3(Control),the location of malignancies(Ca) were: Floor of mouth-2 patients, Ca buccal mucosa-7 patients,Tongue-3 patients Upper alveolus-3 patients, lower alveolus-2 patients, soft palate-3 patients ,maxillary antrum-no patients **(Table 4)**. The TNM staging was done for group 3.The number of patients with TNM staging were: Stage I:No patient, Stage II:2 patients, Stage III: No patient, Stage IVa:17 patients, Stage IVb: 1 patient(**Table 5**).

In The Department of Oral Medicine and Radiology, Tamilnadu Govt. Dental College & Hospital, Chennai-600003, the sample subjects underwent complete extra-oral and intra-oral examinations.

Armamentarium required for clinical examination (Fig 1):

Mouth mirror

Probe

Tweezer

Mask and glove

Good illumination.

Clinical examination of the oral cavity and the surrounding structures were done and the clinical findings recorded in a structured proforma designed for the study. Intra oral (IOPAs and Occlusal views of Maxilla and Mandible) and extra oral radiographs (PNS view, OPG, Lateral and Anteropostreior skull views etc) were taken at the sites of the lesion to find out any erosion of alveolar bone ,extent of the lesion and any other associated lesions. Preliminary laboratory investigations including complete blood investigations like Total count, differential counts of WBC's, RBC count, hemoglobin level, bleeding and clotting times, the peripheral smear etc were done. Patients were referred to the ICTC for test for HIV .All the findings were recorded in the structured proforma designed for the study. The staging of the malignancy was done based on the TNM staging system.

The patients were given habit counselling regarding stopping of the habit of Tobacco usage both in smoking and

chewing form. The patients were motivated for cessation of the habit.

The patients were advised on basic oral hygiene Oral prophylaxis was done. Conservative management with restorations were done for amenable teeth. Extractions of necessary teeth with poor prognosis with poor periodontal condition and in the field of radiation were done. The patients were then referred to the Dept of Radiation Oncology, Government General Hospital, Chennai-600003, for Radiotherapy.

Inclusion criteria:

1. 60 individuals who were planned to undergo a minimum of 6weeks of radiation treatment for oral malignancy were selected.
2. Males and nonpregnant female subjects 30-70 years old who were clinically and histopathologically diagnosed with oral malignancies, who were scheduled to receive a total external beam RT dose of atleast 6000cGy via a megavoltage treatment with a cobalt -60 teletherapy unit were eligible for the study.

Exclusion criteria:

1. Patients not willing to participate in the study

2. Patients having systemic illnesses (for eg: Diabetes, Hypertension etc)
3. Patients with prior history of radiotherapy for any malignancies and patients with concurrent chemotherapy.
4. Patients having residual oral or pharyngeal mucositis from previous radiotherapy or chemotherapy.
5. Patients taking analgesics for other medical conditions
6. Patients with history of allergy to honey or benzydamine hydrochloride
7. Pregnant patients.

PROCEDURE

All the patients,included in the study were explained about the study.Informed consent for the study to be carried out on the patients were obtained both verbally and in written ,both in local language and in english Cobalt- 60 equipment **(Fig 2)** was used for radiation treatment.The equipment consists of a cylinder of diameter 2 cm., height 5 cm., and is positioned in the Cobalt Unit with the circular end facing the patient. Cobalt-60 Gamma radiation typically has energy of about 1.2 MV, D-max being 0.5 cm. and a percentage depth of 55% at 10 cm. Cobalt units with low energy of gamma rays are used for treatment of head and neck cancers.

Conventional fractionated radiation was delivered to the tumour volume at a dose rate of 2 Gy per fraction,treating five fractions per week to a total period of 6-7 weeks. The weekly dosage was 10 Gy(Gray).The total dosage given varied from 60 to 70 Gy(6000 to 7000 cGy).

Radiation source, modality,field size,treatment areas,total planned dose,number of fractions,days of irradiation were recorded in the structured proforma prepared for the study. Assessment of tumour response and complication development were monitored weekly at the radiotherapy review clinic. Baseline liver function tests, Kidney function tests and Blood sugar levels were assessed before the start of the study and the end of the study.

Armamentarium(Study materials) used in our study are (Fig 4)

1. Pure natural honey – Brand name: Dabur honey
2. 0.15%w/v Benzydamine hydrochloride – Brand name: Tantum oral rinse.
3. 0.9% Normal saline.

Patients were divided into three groups by simple random sampling.

Each group comprised of 20 patients.

GROUP I:

In the group I, the patients were instructed to take 20 ml of pure natural honey (**Fig 5(a)**)15 minutes before radiotherapy. They were instructed to slowly rinse the honey in their mouths, swish it around for 5 minutes duration, so as to make the honey in contact of the oral mucosa and slowly swallow so as to make contact with the pharyngeal mucosa (**Fig 5(b)**). The patients were exposed to therapeutic radiation. After 15 mins of this, again 20 ml of pure natural honey is given to the patient to be followed as before.After 6 hours of the radiotherapy, the patients were instructed to again rinse and swallow with 20 ml of pure natural honey and advised to be followed as before.

GROUP II :

Patients were instructed to rinse with 15ml of 0.15% benzydamine Hcl without dilution (**fig 6(a)**),for 5 mins duration,15 minutes before and 15 mins after RT. The patient should be instructed to keep the rinse in contact with the oral mucosa for atleast 5 minutes duration, and then spit it out (**Fig 6(b)**). The patients were exposed to therapeutic radiation. After 15 mins of this, the patients were asked again to rinse with 15 ml of 0.15%w/v benzydamine hydrochloride , for 5 minutes duration and then spit it out. After 6 hours of the radiotherapy, the patients were instructed to again rinse with 15

ml of 0.15% w/v benzydamine hydrochloride for a duration of 5 minutes and then spit it out.

GROUP III(Control group) :

Patients were instructed to rinse with 20ml of 0.9%w/v Normal saline (**Fig 7(a)**)for 5 mins duration. The patient should be instructed to keep the rinse in contact with the oral mucosa for atleast 5 minutes duration, and then spit it out (**Fig 7(b)** .

The patients were exposed to therapeutic radiation. After 15 mins of this, the patients were asked again to rinse with 20 ml of 0.9% Normal saline, for 5 minutes duration and then spit it out. After 6 hours of the radiotherapy, the patients were instructed to again rinse with 20ml 0.9%w/v of normal saline for a duration of 5 minutes and then spit it out.

GRADING OF MUCOSITIS:

All the patients were clinically examined from day 1 of RT, throughout the RT regimen and two weeks after completion of RT for development of oral mucositis.

The complete oral examinations were done everyday for the patients in the three groups. The clinical grading of mucositis was done according to WHO Mucositis Grading.

The onset of mucositis, clinically seen as erythema and soreness without ulceration and without any problems of

alimentation, was noted for every patient as Grade I.The day of onset of grades II, III,and IV were noted during the course of radiotherapy.

After the radiotherapy treatment schedule, the patients were clinically examined for the gradings of mucositis at the end of 1st week after radiotherapy and end of 2nd week after radiotherapy.The findings were recorded in the structured proforma.

STANDARDISATION:

1. Pure natural honey- Brandname: Dabur Honey.

The pure natural honey used in our study was Dabur Honey. Honey is the by-product of flower nectar and the upper aero-digestive tract of the honeybee,which become concentrated by the dehydration process inside the bee-hive. They contain moisture, fructose, glucose, sucrose, maltose and other compounds, along with trace elements. Pure natural honey is ubiquitous, cheap, and natural, and exhibits antibacterial, analgesic and tissue nutritive factors to stimulate re-epithelialisation in the damaged mucosa. Honey has been found effective in burn wounds, oral infections and acceleration of surgical wound healing.

Honey has anti-bacterial properties and enhances epithelialization, thereby improving wound healing. We have

used natural honey (Dabur honey)for the treatment of radiation mucositis to enhance epithelialization of the mucosa, thereby reduce morbidity.

Standardisation of honey:

a. The pure natural honey used for the study was Dabur Honey.
b. The Dabur honey conforms strictly with all statutory requirements of Agmark,the PFA and the International norms for purity.
c. Dabur honey scored the highest on the main parameters of honey purity in recent analysis of all the Indian brands and also obtained ASHCO certification for quality and HACCP (Hazard Analysis and Critical Control Points) certification.

QUALITY CONTROL OF DABUR HONEY:

1. Dabur honey sourced selectively from the Himalayas, the Nilgiris and the Sunderbans forests
2. 2.Collection process follows stringent quality checks to ensure even raw unfiltered honey is of best quality
3. .Entire process is mechanized and untouched by hands to ensure hygienic conditions

2.0.15%w/v Benzydamine hydrochloride- Brand name : Tantum oral rinse

The 0.15% w/v benzydamine hydrochloride used in the study was Tantum soral rinse. Tantum is benzydamine oral rinse containing 0.15% Benzydamine Hcl a non-steroidal anti-inflammatory agent in a flavoured aqueous base with 10% alcohol.It is available in 120 ml bottles.The cost is 35INR. Tantum oral rinse is used to relieve pain and inflammation associated with sore throats, mucositis, stomatitis periodontal surgery and with mouth sores caused by radiation therapy. Prompt relief from symptoms are seen immediately after rinse or gargle.

3.0.9%w/v Normal saline:

The saline used in the study was 0.9% w/v normal saline. Normal saline solution is prepared by adding approximately 1 tsp of table salt to 32 oz of water. It contains no antimicrobial agents. The nominal pH is 5.5 (4.5 to 7.0). 0.9% Sodium Chloride Injection contains 9 g/L Sodium Chloride,) with an osmolarity of 308 mOsmol/L (calc). It contains 154 mEq/L sodium and 154 mEq/L chloride.

INSTITUTIONAL ETHICAL COMMITTEE

Tamil Nadu Government Dental College and Hospital, Chennai-3

Telephone No : 044 2534 0343
Fax : 044 2530 0681

R.C.No. 0431/DE/2010 Date:01.06.2010

Title of the Work : Evaluating the Effectiveness of Topical Application of Pure Natural Honey and 0.15% Benzydamine Hydrochloride on Radiation Induced Mucositis

Principal Investigator: Dr.N.Balaji, IIIrd Year PG student

Department : Dept of Oral Medicine & Radiology
Tamil Nadu Govt:Dental College and Hospital, Chennai-3

The request for an approval from the Institutional Ethical Committee (IEC) was considered for the following on the IEC meeting held on 22.04.2010 at the Principal's Chambers, Tamil Nadu Government Dental College & Hospital, Chennai-3.

"Advised to Proceed with the study"

The Members of the Committee, the Secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the Principal Investigator.

The Principal Investigator and their team are directed to adhere the guidelines given below:

1. You should get detailed informed consent from the patients participating and maintain confidentiality.
2. You should carry out the work without detrimental to regular activities as well as without extra expenditure to the Institution or Government.
3. You should inform the IEC in case of any change of study procedure and investigation or guide.
4. You should not deviate from the area of work for which you have applied for ethical clearance.
5. You should inform the IEC immediately in case of any adverse events or serious adverse reactions. You should abide to the rules and regulations of the Institution.
6. You should complete the work within the specific period and if any extension of time is required. You should apply for permission again and do the work.
7. You should submit the summary of the work to the Ethical Committee on completion of the work.
8. You should not claim funds from the Institution while doing the work or on completion.
9. You should understand that the members of IEC have the right to monitor the work with prior intimation.
10. Your work should be carried out under the direct supervision of your Guide Professor.

SECRETARY

CHAIRMAN

DECLARATION

TITLE OF DISSERTATION	**EVALUATING THE EFFECTIVENESS OF TOPICAL APPLICATION OF PURE NATURAL HONEY AND BENZYDAMINE HYDROCHLORIDE ON RADIATION - INDUCED MUCOSITIS**
PLACE OF THE STUDY	Department of Oral Medicine & Radiology Tamilnadu Government Dental College and Hospital, Chennai-3 and Department of Radiation oncology, Govt. Gen. Hospital, Chennai – 3.
DURATION OF THE COURSE	**3 YEARS**
GUIDE & HEAD OF THE DEPARTMENT	**Dr. S. JAYACHANDRAN M.D.S., Prof and Head, Dept of Oral Medicine and Radiology.**

I hereby declare that no part of dissertation will be utilized for gaining financial assistance / any promotion without obtaining prior permission of the Principal, Tamilnadu Government Dental College and Hospital, Chennai-3. In addition, I declare that no part of this work will be published either in print or in electronic media without the Guide who has been actively involved in dissertation. The author has the right to preserve for publishing of the work solely with the prior permission of the Principal, Tamilnadu Government Dental College and Hospital, Chennai-03.

Guide & Head of the Department Signature of the candidate

INFORMED CONSENT FORM

STUDY TITLE:

EVALUATING THE EFFECTIVENESS OF PURE NATURAL HONEY AND 0.15 % BENZYDAMINE HYDROCHLORIDE ON RADIATION -INDUCED MUCOSITIS

Name: **O.P.No:**

Address: **Serial No:**

Tel. no: **Age / Sex:**

I, _____ age ___ years Exercising my free power of choice, hereby give my consent to be included as a participant in the study "Evaluating the Effectiveness of Pure natural Honey and 0.15 % Benzydamine hydrochloride on Radiation – induced mucositis"

I agree to the following:

- I have been informed to my satisfaction about the purpose of the study and study procedures including investigations to monitor and safeguard my body function.
- I agree to cooperate fully and to inform my doctor immediately if I suffer any unusual symptom.
- I have informed the doctor about all medications I have taken in the recent past and those I am currently taking and other systemic illness that I have.

Name of the patient Name of the investigator

Evaluating the Effectiveness of Pure natural Honey and 0.15% Benzydamine Hydrochloride on Radiation – induced Mucositis.

HISTORY PROFORMA

Serial no.: Dental O.P. No:

Name: Date:

Address: Age / Sex:

Occupation: Tel. no:

Income:

Chief Complaints with Duration:

Past Medical history:

Past Surgical History:

Dental history:

Personal History:

GENERAL EXAMINATION:

Nutritional Status: Good/ Moderate / Poor

HEAD AND NECK EXAMINATION:

Extra – Oral Examination:

CERVICAL LYMPH NODE EXAMINATION:

Intra-Oral Examination:

Oral hygiene: Poor /Fair /Good

Teeth: Mobile: Pain: Lost:

Type of Lesion:

Extension:

Size:

Associated /Preexisting / Precancerous conditions / Lesion:

RADIOGRAPHIC FINDINGS:

CLINICAL STAGING: T….N….M….

STAGE GROUPING:

INVESTIGATIONS:

Group 1 / 2 / 3 :

WHO Mucositis Grading :

Onset of Grade 1 (day) :

Onset of Grade 2 (day) :

Onset of Grade 3 (day) :

Onset of Grade 4 (day) :

Post RT end of First Week (Grade) :

Post RT end of Second Week (Grade) :

Fig 1 : Armamentarium for Clinical Examination

ORAL SQUAMOUS CELL CARCINOMA

	
Squamous Cell Carcinoma of Lower Alveolus	**Squamous Cell Carcinoma of Buccal Mucosa**
	
Squamous Cell Carcinoma of Tongue	**Squamous Cell Carcinoma of Buccal Mucosa**

Fig 2: Oral Squamous cell Carcinoma

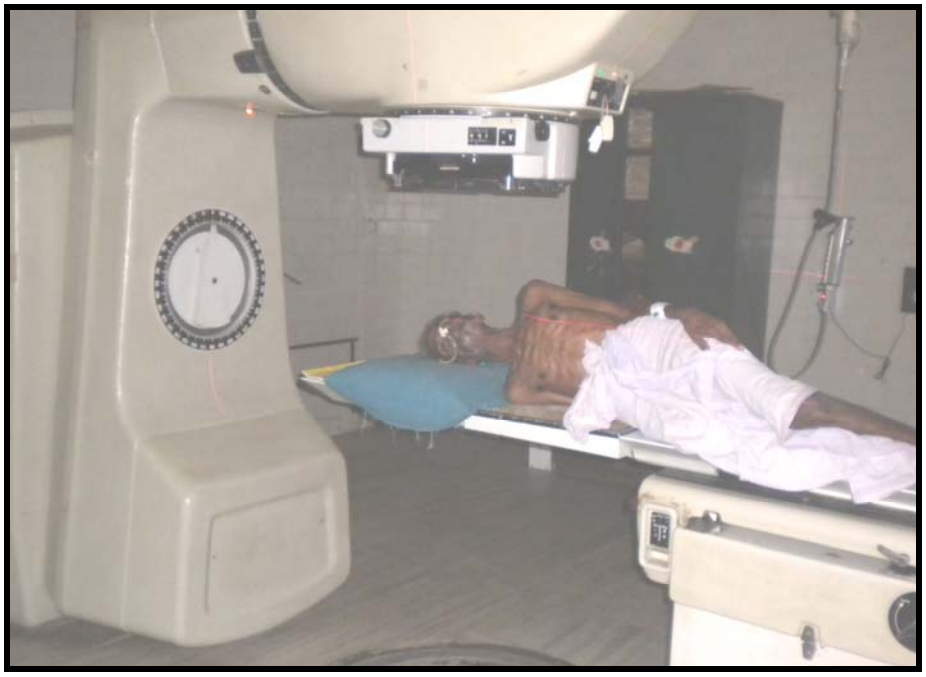


Fig 3 : ORAL CANCER PATIENT ON RADIATION TREATMENT (CO^{60} equipment)

STUDY MATERIALS

Pure Natural Honey

0.15%Benzydamine Hcl

0.9% w/v Normal Saline

Fig 4

GROUP II

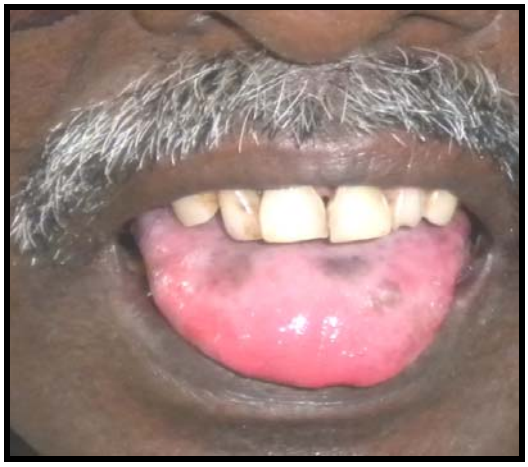


Fig 14:Grade 1 (Erythema and Soreness)

Fig 15 : Grade 2 (Erythema and Ulceration)

Fig 16 : Grade 3 (Erythema, Ulceration, Inability to take solid foods)

Fig 17 : Grade 4 (Erythema, Ulceration, Alimentation not possible)

Fig 18: Post RT end of 1st Week (Grade 2)

Fig 19 : Post RT end of 2nd Week (Grade 1)

GROUP I

Fig 8: Grade 1 (Erythema and Soreness)

Fig 9 : Grade 2 (Erythema and Ulceration)

Fig 10: Grade 3 (Erythema, Ulceration, Inability to take solid foods)

Fig 11 : Grade 4 (Erythema, Ulceration, Alimentation not possible)

Fig 12 : Post RT end of 1st Week (Grade 1)

Fig 13 : Post RT end of 2nd Week (Grade 1)

GROUP III

Fig 20 : Grade 1 (Erythema and Soreness)

Fig 21 : Grade 2 (Erythema and Ulceration)

Fig 22: Grade 3 (Erythema, Ulceration, Inability to take solid foods)

Fig 23 : Grade 4 (Erythema, Ulceration, Alimentation not possible)

Fig 24 : Post RT end of 1st Week (Grade 3)

Fig 25 : Post RT end of 2nd Week (Grade 2)

TOPICAL APPLICATION OF STUDY MATERIALS

Fig 5(a): Pure Natural Honey(20 ml)

Fig 5(b): Topical application of pure natural honey

Fig 6(a) : 0.15% w/v Benzydamine Hydrochloride(15 ml)

Fig 6(b) : Topical application of 0.15% w/v Benzydamine Hydrochloride

Fig 7(a) : 0.9% w/v Normal Saline(15 ml)

Fig 7(b) : Topical application of 0.9% w/v Normal Saline

TABLE 2 (AGE IN YEARS)

	N	Mean	p Value
Group I	20	49.50	0.198
Group II	20	54.00	
Group III	20	55.55	
Total	60	53.02	

TABLE 3
(GENDER DISTRIBUTION AMONG GROUPS)

			Group			Total (n=60)
			Group I (n=20)	Group II (m=20)	Group III (n=20)	
SEX	MALE	Count	11	15	15	41
		% within Group	55.0%	75.0%	75.0%	68.3%
	FEMALE	Count	9	5	5	19
		% within Group	45.0%	25.0%	25.0%	31.7%
TOTAL		Count	20	20	20	60
		% within Group	100.0%	100.0%	100.0%	100.0%

TABLE 4

SITE OF ORAL CANCER

	Buccal mucosa	Tongue	Upper alveolus	Lower alveolus	Floor of mouth	Maxillary antrum	soft palate	TOTAL (n=20)
Group 1	6	4	3	2	1	1	2	20
Group 2	5	3	4	4	0	1	2	20
Group 3	7	3	3	2	2	0	3	20

TABLE 5

TNM STAGING OF ORAL CANCER

	Stage I	Stage II	Stage III	Stage IV a	Stage IV b	Stage IV c	Total
Group 1 (Honey)	0	1	1	16	2	0	20
Group 2 (Tantum)	0	1	0	17	2	0	20
Group 3 (Saline)	0	2	0	17	1	0	20

TABLE 6

(ONSET OF GRADES I,II,III,& IV)

GRADE	GROUP	N	MEAN (in days)
GRADE 1	Group I	20	13.95
	Group II	20	11.85
	Group III	20	11.50
	Total	60	12.43
GRADE 2	Group I	20	18.90
	Group II	20	16.15
	Group III	20	16.10
	Total	60	17.05
GRADE 3	Group I	20	25.25
	Group II	20	21.10
	Group III	20	21.35
	Total	60	22.57
GRADE 4	Group I	10	33.80
	Group II	19	26.74
	Group III	20	26.35
	Total	49	27.53

TABLE 8

	N	MEAN (in weeks)	P VALUE
Group I	**20**	**6.275**	
Group II	**20**	**6.175**	
Group III	**20**	**6.325**	**0.200**
Total	**60**	**6.258**	

(DURATION OF RADIOTHERAPY)

TABLE 7

(MINIMUM,MAXIMUM AND MEAN DAYS OF ONSET OF GRADES I,II,III,& IV IN GROUPS)

			SEX					
			Male			Female		
			Minimum (in days)	Maximum (in days)	Mean (in days)	Minimum (in days)	Maximum (in days)	Mean (in days)
Group	**Group I**	On set	12	16	13.91	12	19	14.00
		Grade 2	14	21	18.18	17	27	19.78
		Grade 3	16	34	24.45	22	36	26.22
		Grade 4	30	38	35.50	25	36	30.50
	Group II	On set	10	15	11.73	12	13	12.20
		Grade 2	14	21	16.20	15	18	16.00
		Grade 3	18	27	21.40	19	22	20.20
		Grade 4	23	36	27.43	23	26	24.80
	Group III	On set	10	14	11.67	10	13	11.00
		Grade 2	14	18	15.93	15	19	16.60
		Grade 3	17	24	21.20	20	24	21.80
		Grade 4	24	29	26.33	24	30	26.40

TABLE 9

(COMPARISON OF ONSET OF GRADES I, II, III IV BETWEEN GROUPS)

Grades	(I) Group	(J) Group	Mean Difference (I-J) in Days	P value
Grade 1	Group I	Group II	2.10(*)	.000
		Group III	2.45(*)	.000
	Group II	Group I	-2.10(*)	.000
		Group III	.35	.679
	Group III	Group I	-2.45(*)	.000
		Group II	-.35	.679
Grade 2	Group I	Group II	2.75(*)	.000
		Group III	2.80(*)	.000
	Group II	Group I	-2.75(*)	.000
		Group III	.05	.997
	Group III	Group I	-2.80(*)	.000
		Group II	-.05	.997
Grade 3	Group I	Group II	4.15(*)	.000
		Group III	3.90(*)	.001
	Group II	Group I	-4.15(*)	.000
		Group III	-.25	.965
	Group III	Group I	-3.90(*)	.001
		Group II	.25	.965
Grade 4	Group I	Group II	4.66(*)	.001
		Group III	5.05(*)	.000
	Group II	Group I	-4.66(*)	.001
		Group III	.39	.919
	Group III	Group I	-5.05(*)	.000
		Group II	-.39	.919

TABLE10
(POST RT END OF FIRST WEEK)

	Grade		Group			Total
			Group I	Group II	Group III	
Post RT end of 1st week	1	Count	4	0	0	4
		% within Post RT 1st week	100.0%	.0%	.0%	100.0%
	2	Count	14	10	4	28
		% within Post RT 1st week	50.0%	35.7%	14.3%	100.0%
	3	Count 44	2	10	11	23
		% within Post RT 1st week	8.7%	43.5%	47.8%	100.0%
	4	Count	0	0	5	5
		% within Post RT 1st week	.0%	.0%	100.0%	100.0%
Total		Count	20	20	20	60
		% within Post RT 1st week	33.3%	33.3%	33.3%	100.0%

TABLE 11
(POST RT END OF SECOND WEEK)

	Grade		Group			Total
			Group I	Group II	Group III	
Post RT end of 2nd week	0	Count	3	0	0	3
		% within Post RT 1st week	100.0%	.0%	.0%	100.0%
	1	Count	15	12	5	32
		% within Post RT 1st week	46.9%	37.5%	15.6%	100.0%
	2	Count	2	7	9	18
		% within Post RT 1st week	11.1%	38.9%	50.0%	100.0%
	3	Count	0	1	6	7
		% within Post RT 1st week	.0%	14.3%	85.7%	100.0%
Total		Count	20	20	20	20
		% within Post RT 1st week	33.3%	33.3%	33.3%	33.3%

MASTER CHART

TABLE 1 (Group 1)

S. No.	Name	Age	Sex	Site	TNM Staging	Dose	Duration of RT (in days)	grade 1 (Day)	grade 2 (Day)	grade 3 (Day)	grade 4 (Day)	post RT 1st (grade)	post RT 2nd (grade)
1	Balan	55	M	Buccal Mucosa	IV a	60	6	12	15	20		2	1
2	Kannan	54	M	Tongue	IV a	66	6.5	14	20	24	38	2	1
3	Thangavel	65	M	Upper Alveolus	IV a	66	6.5	13	17	27	37	2	1
4	Ramajayam	40	M	Lower alveolus	IV a	66	6.5	13	17	27	37	2	1
5	Saroja	50	F	Buccal Mucosa	IV a	60	6	14	20	28	36	2	1
6	Ranganathan	40	M	Tongue	IV a	60	6	12	14	16	30	1	1
7	Govindammal	30	F	Floor of mouth	IV a	66	6.5	14	27	31		2	1
8	Jayammal	47	F	Maxillary Antrum	IV b	66	6.5	19	23	36		2	1
9	Krisnaveni	50	F	Soft Palate	IV a	60	6	14	18	24		2	1
10	Kamala	51	F	Buccal Mucosa	IV a	60	6	14	19	24		1	0
11	Saluddin	60	M	Buccal Mucosa	IV a	60	6	15	19	24		1	0
12	Pakkiri	55	M	Tongue	IV a	60	6	16	21	34		2	1
13	Padma	45	F	Upper Alveolus	IV a	60	6	12	17	22	25	3	2
14	Rajabader	48	M	Lower alveolus	III	66	6.5	15	20	24		2	1
15	Venkatesan	35	M	Upper Alveolus	IV a	66	6.5	15	20	25		2	0
16	Jaya	60	F	Tongue	IV a	60	6	12	17	23		2	1
17	Arumugam	40	M	Buccal Mucosa	II	70	7	14	18	24		2	1
18	Vasanta	55	F	Soft Palate	IV a	60	6	13	17	22		1	1
19	Ravindran	45	M	Buccal Mucosa	IV b	66	6.5	14	19	24		2	1
20	Amsavalli	65	F	Soft Palate	IV a	66	6.5	14	20	26		3	2

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TABLE 1 (Group 2)

S. No.	Name	Age	Sex	Site	TNM Staging	Dose	Duration of RT (in days)	grade 1 (Day)	grade 2 (Day)	grade 3 (Day)	grade 4 (Day)	post RT 1st (grade)	post RT 2nd (grade)
21	Ahamed	60	M	Buccal Mucosa	IV a	66	6.5	12	16	23	28	2	1
22	Natrajan	60	M	Tongue	IV a	60	6	15	21	24	36	2	1
23	Chellappan	52	M	Upper Alveolus	IV a	66	6.5	12	16	20	24	3	2
24	Lakshmi	55	F	Buccal Mucosa	IV a	60	6	12	16	19	24	3	1
25	Pazhanivel	45	M	Tongue	IV a	66	6.5	13	17	27	32	2	1
26	Mary	60	F	Lower alveolus	IV a	60	6	12	15	19	23	2	1
27	Ramaswamy	60	M	Maxillary Antrum	IV a	60	6	11	16	21	27	3	1
28	Manibarathi	61	M	Soft Palate	IV a	60	6	10	15	18		3	2
29	Sekar	40	M	Buccal Mucosa	II	60	6	11	14	20	25	2	1
30	Shahul	34	M	Buccal Mucosa	IV a	60	6	11	14	21	25	2	1
31	Masanamuthu	66	M	Tongue	IV a	66	6.5	10	17	23	30	3	2
32	Kesavulu	55	M	Upper Alveolus	IV a	60	6	11	14	18	25	2	2
33	Durairaj	62	M	Lower alveolus	IV a	66	6.5	12	17	23	30	3	1
34	Gopal	60	M	Upper Alveolus	IV a	60	6	12	17	22	26	2	1
35	Umabaskar	39	M	Buccal Mucosa	IV b	60	6	12	16	21	27	3	2
36	Thangappan	68	M	Upper Alveolus	IV a	60	6	13	18	20	26	3	3
37	Suguna	40	F	Lower alveolus	IV b	60	6	13	18	22	26	2	1
38	Rasayyan	63	M	Lower alveolus	IV a	66	6.5	11	15	20	23	3	2
39	Lakshmiammal	45	F	Soft Palate	IV a	60	6	12	15	20	25	3	2
40	Saraswathi	55	F	Upper Alveolus	IV a	66	6.5	12	16	21	26	2	1

MASTER CHART

TABLE 1 (Group 3)

S. No.	Name	Age	Sex	Site	TNM Staging	Dose	Duration of RT (in days)	grade 1 (Day)	grade 2 (Day)	grade 3 (Day)	grade 4 (Day)	post RT 1st (grade)	post RT 2nd (grade)
41	Pazhanivelu	70	M	Buccal Mucosa	IV a	66	6.5	11	16	21	26	3	2
42	Subramani	45	M	Buccal Mucosa	IV a	60	6	12	16	20	24	3	2
43	Manickam	65	M	Tongue	IV a	60	6	12	17	24	29	3	2
44	Rajeswari	49	F	Upper Alveolus	IV a	60	6	11	15	20	24	2	1
45	Gowri	45	F	Upper Alveolus	IV a	60	6	10	15	20	24	2	1
46	Sigamani	60	M	Lower alveolus	IV a	66	6.5	11	14	19	24	3	2
47	Sadagopan	53	M	Floor of mouth	IV a	66	6.5	12	16	21	26	3	3
48	Munusamy	50	M	Soft Palate	IV b	66	6.5	12	15	20	24	3	2
49	Babu	42	M	Buccal Mucosa	IV a	66	6.5	14	18	24	28	4	3
50	Muniyan	57	M	Tongue	IV a	66	6.5	11	15	20	25	2	1
51	Perumal	67	M	Upper Alveolus	IV a	60	6	12	18	23	29	3	1
52	Damodaran	57	M	Lower alveolus	IV a	60	6	13	14	17	26	3	2
53	Rajendran	45	M	Tongue	IV a	66	6.5	10	14	20	27	4	3
54	Mangammal	65	F	Buccal Mucosa	II	66	6.5	13	17	21	24	3	2
55	Lakshmi	50	F	Buccal Mucosa	IV a	66	6.5	10	19	24	30	2	1
56	Sekar	42	M	Soft Palate	IV a	60	6	10	15	21	25	4	3
57	Chandran	55	M	Floor of mouth	II	66	6.5	11	16	21	26	3	2
58	Masanam	65	M	Buccal Mucosa	IV a	66	6.5	12	18	24	28	4	3
59	Kamakshi	46	F	Soft Palate	IV a	66	6.5	11	17	24	30	3	2
60	Gajendrababu	53	M	Buccal Mucosa	IV a	66	6.5	12	17	23	28	4	3

RESULTS AND ANALYSIS

The present study was directed to evaluate the day of onset of radiation-induced mucositis (Grade I of WHO mucositis Grading) and the severity, the progression to grades II, III, IV, in three groups during the course of radiotherapy for malignancy of oropharynx, of a total dose of radiation varying between 60 to 70 Gys, with a fractionation dose varying between 200 to 300 Gy per day in the range of 6 to 7 weeks.

60 patients, who were to undergo radiotherapy for malignancy of oropharynx **(Fig 7)** ,with field involving the oral and associated region selected for the study, there were 41 males and 19 females**.** The minimum age of male was 34 years and the maximum age was 70 years. The minimum age of female was 30 years and the maximum age was 65 years**. (Table-1,3)** These patients were planned for radiotherapy.

Cobalt- 60 equipment was used for radiation treatment. The equipment consists of a cylinder of diameter 2 cm, height 5 cm. and is positioned in the Cobalt Unit with the circular end facing the patient. Cobalt-60 Gamma radiation typically has energy of about 1.2 MV, D-max being 0.5 cm. and a percentage depth of 55% at 10 cm.

Cobalt units with low energy of gamma rays are used for treatment of head and neck cancers. Conventional fractionated

radiation was delivered to the tumour volume at a dose rate of 2 Gy per fraction, treating five fractions per week to a total period of 6-7 weeks. The weekly dosage was 10 Gy(Gray).The total dosage given varied from 60 to 70 Gy(6000 to 7000 cGy Radiation source, modality, field size, treatment areas, total planned dose, number of fractions, days of irradiation were recorded in the structured proforma prepared for the study.

The 60 patients were randomly divided into three groups (**Table-1,3**). All the patients received radiotherapy regimen as planned .The total dose of radiation varied between 60 to 70 Gy with a fractionation dose of 200 to 300 cGy per day in the range of 6 – 7 weeks.

Group 1: 20 patients (n=20)

Group 1 consisted of 11(51%) males and 9(45%) females. (**Table-1,3).** the mean age of both genders in group 1 is 49.50 years**(Table-2)**. The mean duration of RT in the Group 2 was 6.275(+0.3024) **(Table-8)** In the group I, the patients were instructed to take 20 ml of pure natural honey 15 minutes before radiotherapy .They were instructed to slowly rinse the honey in their mouths, swish it around for 5 minutes duration ,so as to make the honey in contact of the oral mucosa and slowly swallow so as to make contact with the pharyngeal mucosa.

The patients were exposed to therapeutic radiation. After 15 mins of this, again 20 ml of pure natural honey is given to the patient to be followed as before. After 6 hours of the radiotherapy, the patients were instructed to again rinse with 20 ml of pure natural honey. The patients take any food for one hour after the application of pure natural honey.

Out of 20 subjects in Group 1, 4 patients developed grade I mucositis on the 12^{th} day,3 patients developed gradeI mucositis on 13^{th} day,8 patients developed on 14^{th} day,3 patients developed mucositis on 15^{th} day,1 patient developed grade I mucositis on 16^{th} day,1 patient developed grade I mucositis on 19^{th} day.

In the maximum number of patients, the onset of mucositis was on the 14^{th} day, totally 8 patients developed mucositis on 14^{th} day. The mean onset of mucositis was on 14^{th} day (Mean 13.95+1.638 days) (**Table 6**).

Out of the 20 subjects,in group 1,1patient developed grade II mucositis on 14^{th} day,1 patient developed grade II on 15^{th} day,5 patients developed grade II on 17^{th} day,2 patients developed grade II on 18^{th} day,3 patients developed gradeII on 19^{th} day,5 patients developed grade II mucositis on 20^{th} day,1 patient developed gradeII on 21^{st} day,1 patient on 23^{rd} day and 1 patient on 27^{th} day(**Table 6**).

The maximum number of patients developed grade II on 17th and 20th days equally. The mean onset of grade II was on 19th day (Mean 18.90+2.827 days). **(Table 6**).

Out of the 20 subjects in group 1,1 patient developed grade III mucositis on 16th day,1 patient developed grade III on 20th day,2 patients developed grade III on 22nd day,1 patient developed gradeIII on 23rd day,7 patients developed grade III on 24th day,1 patient developed grade III on 25th day, 1 patient developed gradeIII on 26th day,2 patients developed grade III on 27th day,1 patient developed grade III on 28th day,1 patient developed grade III on 31st day,1 patient developed grade III on 34th day and 1 patient developd grade III on 26th day. The maximum number of patients (7 patients) developed grade III on 24th day. The mean onset of grade III mucositis was on 25th day (Mean 25.25+4.529 days). **(Table 6**).

Only 6 patients developed grade IV of WHO mucositis grading in Group1.1 patient developed grade IV mucositis on 25th day,1 patient developed grade IV mucositis on 30th day,1 patient developed grade IV mucositis on 36th day,2 patients developed grade IV mucositis on 37th day,1 patient developed grade IV mucositis on 38th day. The maximum number of patients developed grade IV on 37th day .The mean onset is on 34th day(Mean 33.80+4.195 days) **(Table 6)**

After the end of the course of the radiotherapy, the patients were instructed to follow the same procedure, of topical application of pure natural honey, for two weeks duration, after the last dose of RT. The 20 patients in group1 were clinically examined for the gradings of mucositis at the end of 1st week of radiotherapy.

Out of the 20 patients in the Group1,4 patients were having gradeI mucositis,14 patients were having grade II mucositis,2 patients were having grade III mucositis. Grade IV mucositis was seen in no patients. The maximum number of patients were in Grade II mucositis (14 patients out of total 20 in grade I) **(Table10**).

In the post RT end of 2nd week, all the 20 patients were clinically examined for the grade of mucositis. Out of the 20 patients in Group1,3 patients were in grade 0 mucositis at the end of post RT 2nd week.15 patients were having grade 1 mucositis,2 patients were having grade 2 mucositis. Grades 3 &4 were not seen in any patient. **(Table11)**.

Group 2(n=20):

Group 2 had 15(75%) males and 5(25%) females**(Table1,3)**. The mean age of both genders in group II was 54.00**(Table-2).** The RT regimen consisted of a total dose of radiation varying between 60 t0 70 Gy with a fractionation

dose of 200 to 300 cGy per day in the range of 6-7 weeks. The mean duration of RT in the Group 2 was 6.175(0.2447) in weeks. **(Table-9)**

Patients were instructed to rinse with 15 ml of 0.15% benzydamine Hcl without dilution,for 5 minutes duration,15 minutes before and 15 mins after RT. The patient should be instructed to keep the rinse in contact with the oral mucosa for atleast 5 minutes duration, and then spit it out.

The patients were exposed to therapeutic radiation. After 15 mins of this, the patients were asked again to rinse with 15 ml of 0.15%w/v benzydamine hydrochloride , for 5 minutes duration and then spit it out. After 6 hours of the radiotherapy, the patients were instructed to again rinse with 0.15% w/v benzydamine hydrochloride for a duration of 5 minutes and then spit it out. The patients were instructed not to take any food for one hour after the application of benzydamine hydrochloride.

Out of the 20subjects in the group 2, 2 patients developed grade I mucositis on the 10^{th} day,5 patients developed grade I mucositis on the 11^{th} day,9 patients developed grade I mucositis on 12^{th} day,3 patients developed grade I mucositis on 13^{th} day, 1 patient developed grade I mucositis on 15^{th} day. The mean onset was on 12^{th} day (Mean 11.85+1.137 days). **(Table-6)**.

Out of the 20 subjects, 3 patients developed grade II on

14th day,4 patients developed grade II on 15th day,6 patients developed grade II on 16th day,4 patients on 17th day,2 patients on 18th day,1 patient on 21st day. The mean onset of grade II was on 16th day (Mean 16.15+1.663 days) **(Table-6**).

Out of the 20 subjects, 2 patients developed gradeIII on 18th day,2 patients developed grade III on 19th day,5 patients developed grade III on 20th day,4 patients developed grade III on the 21st day,2 patients developed grade III on 22nd day,3 patients developed grade III on the 23rd day,1 patient developed grade III on the 24th day,1 patient developed grade III on the 27th day**(Table-6)**.

The mean onset of grade III in group 2 was on the21st day (Mean 21.10+2.174 days). Out of the 20 subjects in group 2, 19 patients developed Grade IV mucositis. One person did not develop grade IV mucositis. Out of the 19 patients,2 patients developed grade IV on the 23rd day,2 patients developed grade IV on 24th day,4 patients developed grade IV mucositis on the 25th day,4 patients developed grade IV mucositis on the 26th day,2 patients developed grade IV mucositis on 27th day,1 patient developed grade IV mucositis on 28th day,2 patients developed grade IV mucositis on the 30th day,1 patient developed grade IV mucositis on the 32nd day,1 patient developed grade IV mucositis on the 36th day **(Table-6).** The

mean onset of Grade IV in group 2 was on 27th day (Mean 26.74+3.280 days)

In the post RT end of 1st week, all the 20 patients in group 2 were clinically examined for the grade of mucositis. Out of the 20 patients in the group2, grades 0 or 1 were not seen in any of the patients.10 patients were having grade II mucositis.10 patients were with grade III mucositis, no patient was with grade IV mucositis **(Table-10).**

In the post RT end of 2nd week, all the 20 patients in group 2 were clinically examined for the grade of mucositis. Out of the 20 patients in group 2, No patient was with grade 0 mucositis.12 patients were having grade I mucositis.7 patients were with grade II mucositis,1 patient was with grade III mucositis, no patients were seen to have grade IV mucositis.**(Table-11)**

GROUP 3(n=20):

Group3 had 20 patients. There were 15(75%) males and 5(25%) females **(Table 1)**. The mean age of both genders in group 3 was 55.55 years **(Table-3)**. The RT regimen consisted of a total dose of radiation varying between 60 t0 70 Gy with a fractionation dose of 200 to 300 cGy per day in the range of 6-7 weeks. The mean duration of RT in the Group 3 was6.325(0.2447) **(Table-9)**.

Patients were instructed to rinse with 20ml of 0.9%w/v Normal saline for 5 minutes duration. The patient should be instructed to keep the rinse in contact with the oral mucosa for atleast 5 minutes duration, and then spit it out.

The patients were exposed to therapeutic radiation .After 15 mins of this, the patients were asked again to rinse with 20 ml of 0.9%w/v Normal saline, for 5 minutes duration and then spit it out. After 6 hours of the radiotherapy, the patients were instructed to again rinse with 20 ml0.9%w/v of normal saline for a duration of 5 minutes and then spit it out.

Out of the 20 patients in Group 3, 4 patients developed grade I mucositis on the 10^{th} day,6 patients developed grade I mucositis on the 11^{th} day,7 patients developed grade I mucositis on the 12^{th} day,2 patients developed grade I mucositis on the 13^{th} day,1 patient developed grade I mucositis on the 14^{th} day. The mean onset of grade I mucositis in group 3 was on 12^{th} day (Mean 11.50+1.100 days) **(Table-6)**

Out of the 20 subjects in group 3,3 patients developed grade II on the 14^{th} day,5 patients developed grade II on 15^{th} day,4 patients developed grade III on 16^{th} day,4 patients developed grade II on 17^{th} day,3 patients developed grade II on 18^{th} day,1 patient developed grade II on 19^{th} day. The grade II was on 16^{th} day (Mean 16.10+1.483 days) **(Table-6)**

Out of the 20 subjects in Group 3,1 patient developed grade III on 17th day,1 patient developed grade III on 19th day,6 patients developed grade III on 20th day,5 patients developed grade III on 21st day,2 patients developed grade III on 23rd day,5 patients developed grade III on 24th day. The mean onset of grade III in group3 was on 21st day (Mean 21.35+2.007) **(Table-6)**

Out of the 20 subjects in groupIII,all the subjects developed grade IV mucositis.This shows that the control group had a minimal protection against incidence of grade IV mucositis **(Table-6).**

Out of the 20 subjects,6 patients developed grade IV mucositis on the 24th day,2 patients developed grade IV mucositis on the 25th day,4 patients developed grade IV mucositis on the 26th day,1 patient developed grade IV mucositis on the 27th day,3 patients developed grade IV mucositis on 28th day,2 patients developed grade IV mucositis on the 29th day,2 patients developed grade IV mucositis on the 30th day. The mean onset of Grade IV mucositis was on 26th day (Mean 26.35+2.134 days) **(Table-6)**

In the post RT end of 1st week, all the 20 patients in group 3 were clinically examined for the grade of mucositis. Out of the 20 patients in the group3,no patients were with grade

0 and grade I mucositis. 4 patients were having gradeII mucositis.11 patients were with grade III mucositis,[5] patients were with grade IV mucositis **(Table-10).**

In the post RT end of 2nd week, all the 20 patients in group 3 were clinically examined for the grade of mucositis. Out of the 20 patients in group 3, 5 patients were having gradeI mucositis.9 patients were with grade II mucositis,6 patients were with grade III mucositis. None were with grade IV mucositis **(Table-11).**

Comparison of duration of radiotherapy between groups:

The duration of RT in the 60 patients were between 6-7 weeks. The mean duration of RT, in weeks, in groups 1,2,3 were 6.275(+0.3024). 6.175(0.2447), 6.325(0.2447) respectively. The p value is 0.200(Not significant at 5%) that shows that there is no major difference in the duration of RT between the three groups. **(Table-9)**

Comparison of onset (grade 1 of WHO mucositis grading system) between groups :

The mean onset of mucositis(Grade I),clinically seen as Erythema without ulceration with symptoms of soreness in the oral mucosa, in the three groups were noted. The mean onset of mucositis in Group 1 was on 14th day (Mean 13.95+1.638 days) The mean onset of mucositis in Group 2 was on 12th day (Mean

11.85+1.137 days) The mean onset of mucositis in Group 3 was on 14th day (Mean 11.50+1.100 days) The mean difference on onset between Groups 1 & 3 was 2.45 days The p-value between Group1 & 3 was <0.001. The mean difference on onset between Groups 2 & 3 was 0.35 days The p-value between Group2 & 3 was 0.679. The mean difference on onset between Groups 1 & 2 was 2.10 days. The p-value between Group1 & 2 was <0.001**. (Table-8)**

The statistical anlaysis of the days of onset between groups shows that group1 (Pure natural Honey group) has a delayed onset of mucositis in comparison to group2(0.15%benzydamine hydrochloride group) and group 3(0.9% normal saline).

There is no major difference in the onset between groups 2 & 3.Hence, pure natural honey delays the onset of radiation-induced mucositis.

Comparison of onset of grade II (WHO mucositis grading system) between groups:

The mean onset of grade II mucositis,clinically seen as Erythema with ulceration with symptoms of soreness in the oral mucosa, in the three groups were noted. The mean onset of grade II mucositis in Group 1 was on 19th day (Mean 18.90+2.827 days) The mean onset of grade II mucositis in

Group 2 was on 16th day (Mean 16.15+1.663 days) The mean onset of grade II mucositis in Group 3 was on 16th day(Mean 16.10+1.483 days) **(Table-6)** The mean difference on onset of grade II between Groups 1 & 3 was 2.80 days.

The p-value between Group1 & 3 was <0.001. The mean difference on onset of grade II between Groups 2 & 3 was 0.05 days The p-value between Group2 & 3 was 0.997 The mean difference on onset of grade II between Groups 1 & 2 was 2.75 days The p-value between Group1 & 2 was <0.001**(Table-8)**

The statistical anlaysis of the days of onset of grade II between groups shows that group1 (Pure natural Honey group) has a delayed onset of grade II mucositis in comparison to group2(0.15%benzydamine hydrochloride group) and group 3(0.9% normal saline).

There is no major difference in the onset of grade II between groups 2 & 3.Hence, pure natural honey delays the onset grade II of radiation-induced mucositis.

Comparison of onset of grade III (WHO mucositis grading system) between groups :

The mean onset of grade III mucositis, clinically seen as Erythema with ulceration with symptoms of soreness in the oral mucosa, and functional impairment to take solid foods, in the three groups were noted. The mean onset of grade III mucositis

in Group 1 was on 25th day(Mean 25.25+4.529 days) The mean onset of grade III mucositis in Group 2 was on 21st day(Mean 21.10+2.174 days) The mean onset of grade III mucositis in Group 3 was on 21st day(Mean 21.35+2.007 days) **(Table-6)**

The mean difference on onset of grade III between Groups 1 & 3 was 3.90 days. The p-value between Group1 & 3 was <0.001 The mean difference on onset of grade III between Groups 2 & 3 was -0.25 days The p-value between Group2 & 3 was 0.965(Table-7) The mean difference on onset of grade III between Groups 1 & 2 was 4.15 days The p-value between Group1 & 2 was <0.001**(Table-8)**

The statistical anlaysis of the days of onset of grade III between groups shows that group1(Pure natural Honey group) has a delayed onset of grade III mucositis in comparison to group2(0.15%benzydamine hydrochloride group) and group 3(0.9% normal saline).

Comparison of onset of grade IV (WHO mucositis grading system) between groups :

The mean onset of grade IV mucositis, clinically seen as Erythema with ulceration with symptoms of soreness in the oral mucosa,and functional impairment to take solid foods and liquids also(alimentation not possible), in the three groups were noted. The mean onset of grade IV mucositis in Group 1 was on

34^{th} day(Mean 33.80+4.195 days) The mean onset of grade IV mucositis in Group 2 was on 27^{th} day(Mean 26.74+3.280 days) The mean onset of grade IV mucositis in Group 3 was on 26^{th} day(Mean 26.35+2.134 days) **(Table-6)**

The mean difference on onset of grade IV between Groups 1 & 3 was 7.06 days. The p-value between Group1 & 3 was <0.001 The mean difference on onset of grade IV between Groups 2 & 3 was 0.39 days The p-value between Group2 & 3 was 0.919 The mean difference on onset of grade IV between Groups 1 & 2 was 7.45 days The p-value between Group1 & 2 was <0.001**(Table-8)**

The statistical analysis of the days of onset of grade IV between groups shows that group1 (Pure natural Honey group) has a delayed onset of grade IV mucositis in comparison to group2(0.15%benzydamine hydrochloride group) and group 3(0.9% normal saline). Another finding in the present study is that out of 20 patients in group1(pure natural honey group),only 6 patients developed intolerable grade IV mucositis.

Comparison of Post-RT end of 1^{st} week in groups :

In the post RT end of 1^{st} week,grade I was seen in 4 patients(All patients in Group1) at the end of 1^{st} week post radiotherapy. None of the patients in the Groups 2&3 were clinically seen with grade 1. Grade II clinically seen in 28

patients (group 1-14 patients,Group 2-10 patients,group3-4 patients) at the end of 1st week post radiotherapy Grade III clinically seen as erythema and soreness with ulceration and symptoms of inability to take solid foods, were seen in 23 patients (Group 1- 2 patients group2- 10 patients,group3- 11 patients) at the end of 1st week post radiotherapy Grade IV clinically seen as erythema, soreness and ulceration and functional impairment to take solids and liquid diet also (alimentation not possible),was seen in5 patients(Group 3- 5 patients) at the end of 1st week post radiotherapy.

At the end of 1st week post radiotherapy, Out of 20 patients in the Group 1, only 2 patients were with grade III mucositis, compared to 10 patients in the group2 and 11 patients in group 3.This shows the low incidence of grade III in the group 1 compared to groups 2 & 3. Grade IV was seen in 5 patients (Group 3 – 5 patients) **(Table-10)**

Comparison of Post-RT end of 2nd week in groups 1,2&3:

In the post RT end of 2nd week, grade 0 clincally seen as a normal mucosa was seen in 3 patients all from group I. In the post RT end of 2nd week, grade I, was seen in 32 patients(Group1- 15 patients, group 2-12 patients, group 3-5 patients). at the end of 2nd week post radiotherapy Grade II clinically was seen in 18 patients (group 1- 2patients,Group 2-

7 patients,group3- 9 patients) at the end of 2nd week post radiotherapy. Grade III clinically was seen in 7 patients (Group 1- no patients group2- 1 patient,group3- 6 patients.) at the end of 2nd week post radiotherapy None of the patients were of grade IV ,out of the 60 patients at the end of the 2nd week post-radiotherapy.

At the end of 2nd week post radiotherapy, Out of 20 patients in the Group 1 ,no patients were with grade III mucositis, compared to 1 patient in the group2 and 6 patients in group 3**(Table-11)**

The results from the present study reveal that pure natural honey delays the onset of grade 1 of radiation-induced mucositis. The severity of radiation-induced mucositis, assessed by the number of patients with symptomatic grade IV, is also reduced by the use of pure natural honey. Thus, topical application of natural honey is a simple and cost-effective treatment in radiation mucositis.

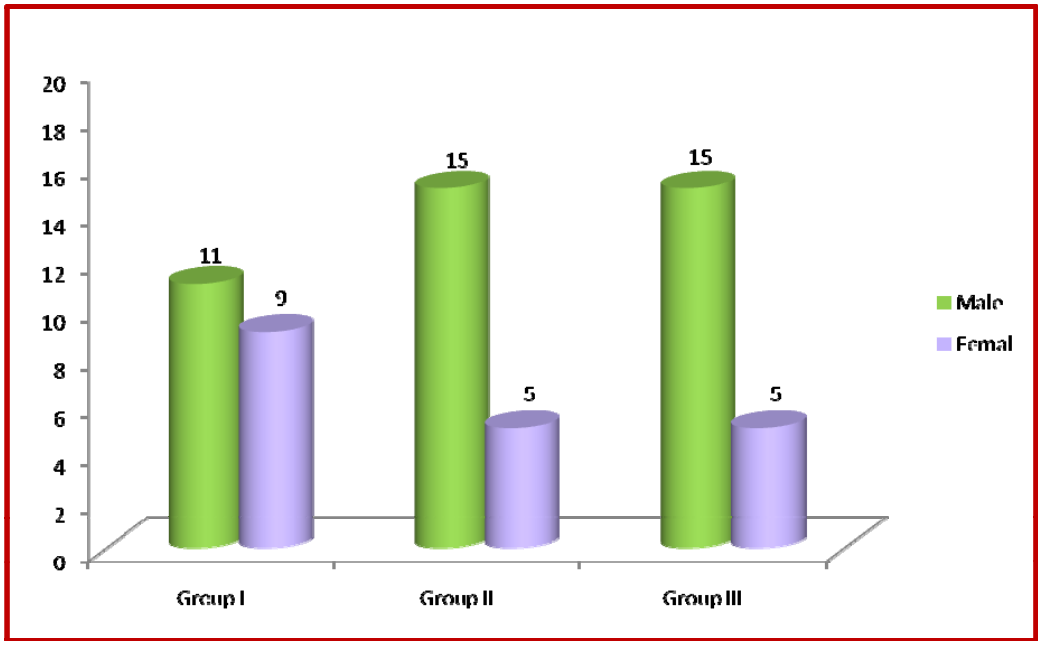

Chart 1 : Age in years

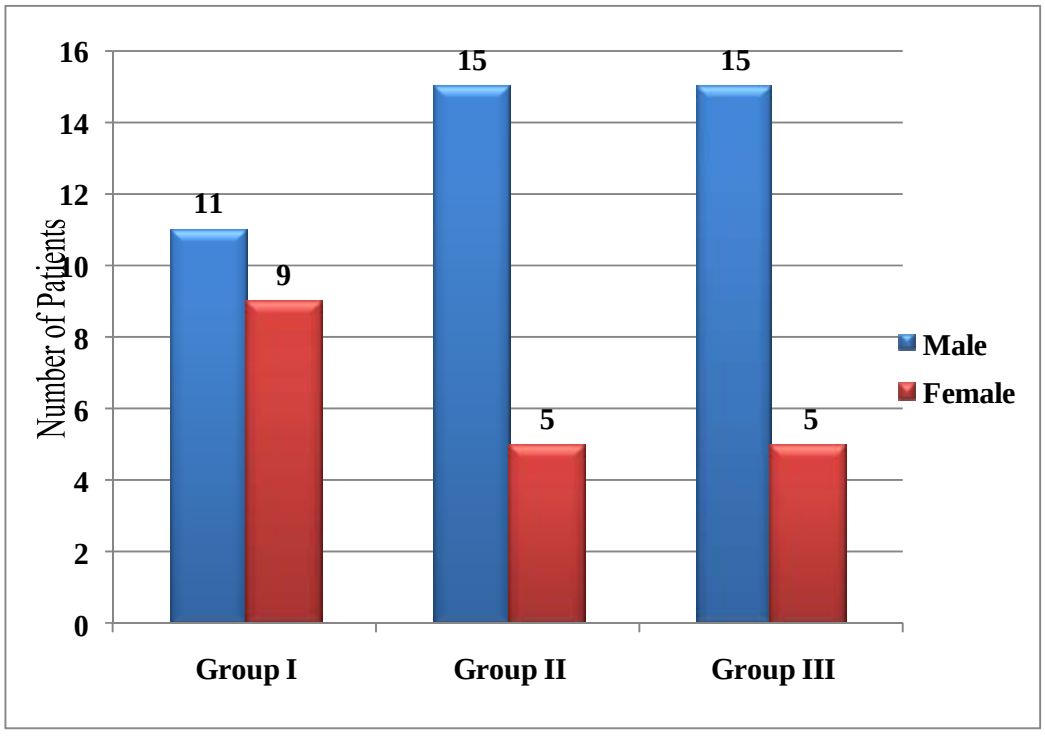

Chart 2 : Gender Distribution Among Groups

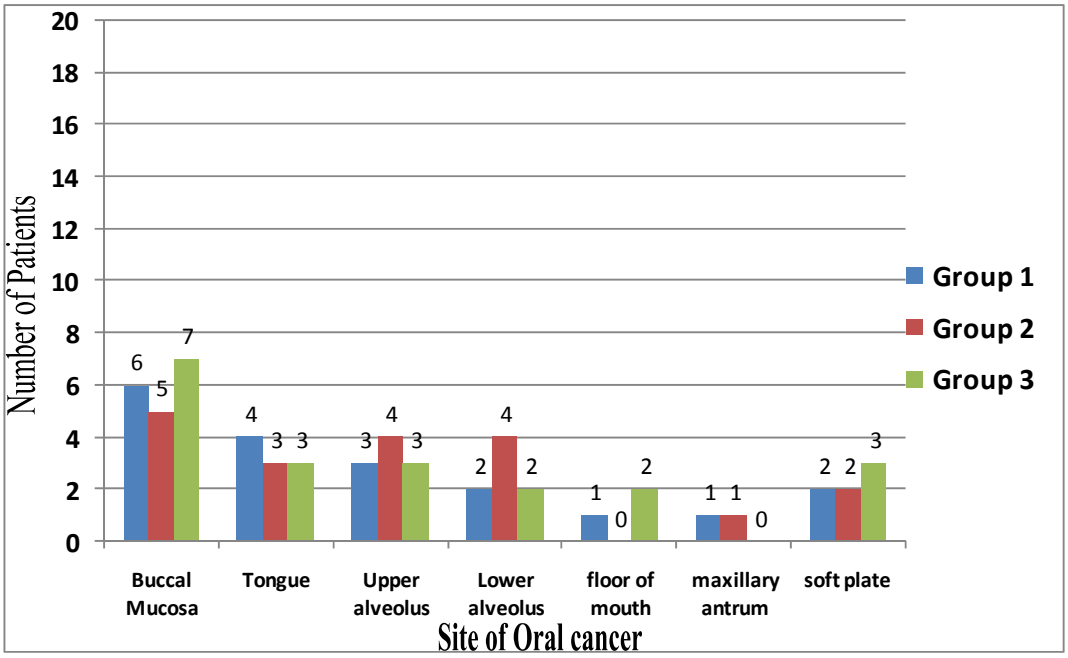

Chart 3 : Site Of Oral Cancer

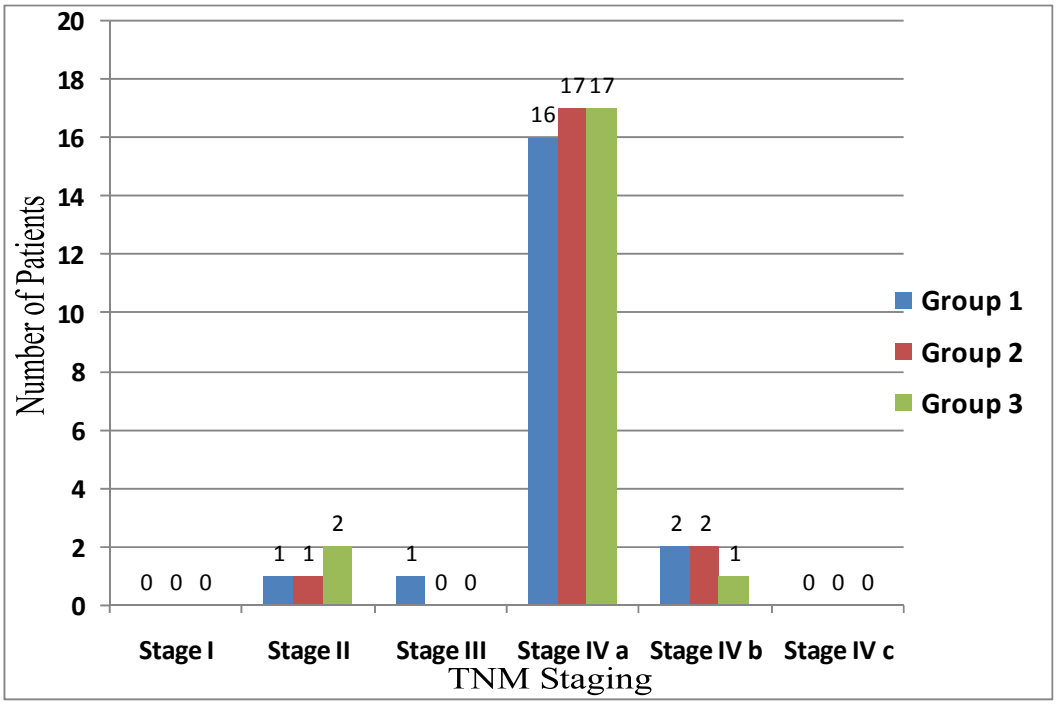

Chart 4 : TNM Staging Of Oral Cancer

Grade I

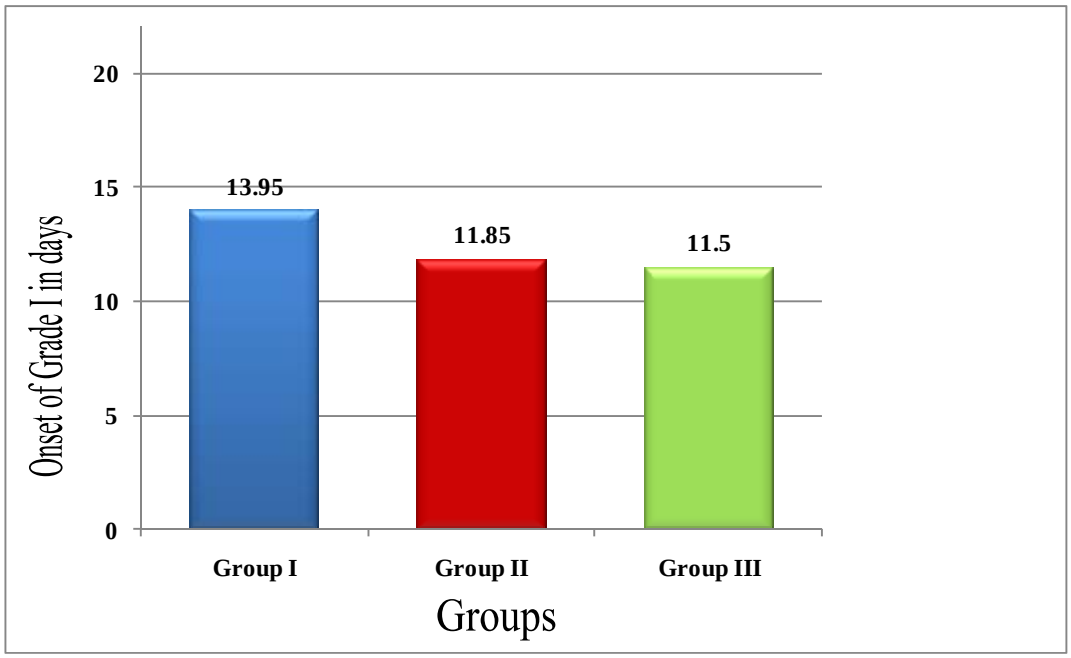

Chart 5 : ONSET OF GRADES I

Grade II

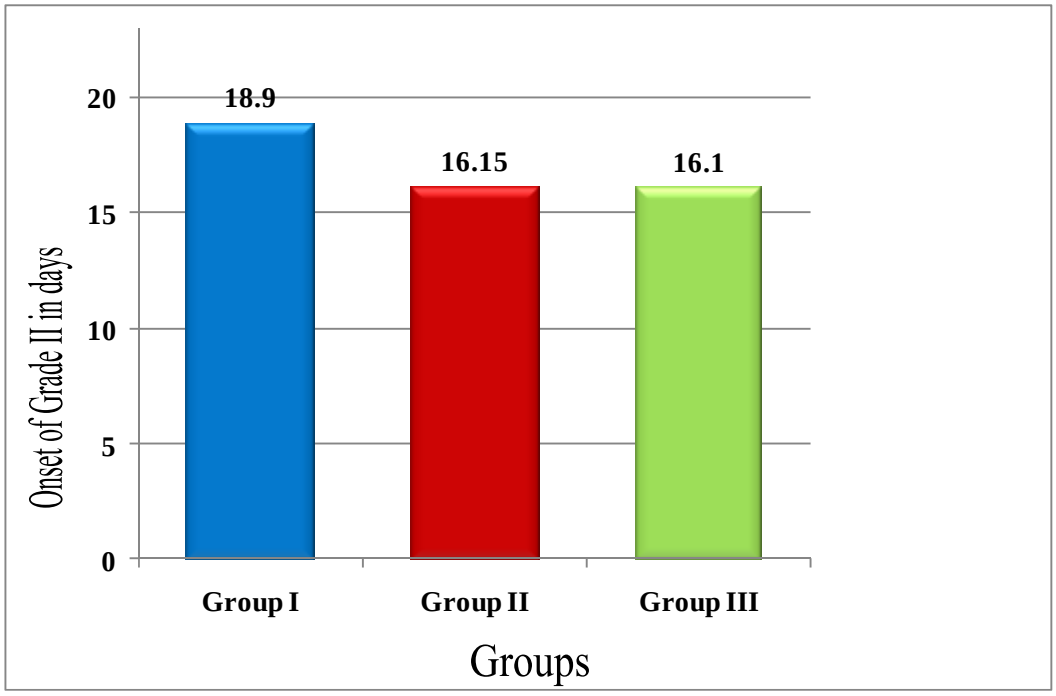

Chart 6 : ONSET OF GRADES II

Grade III

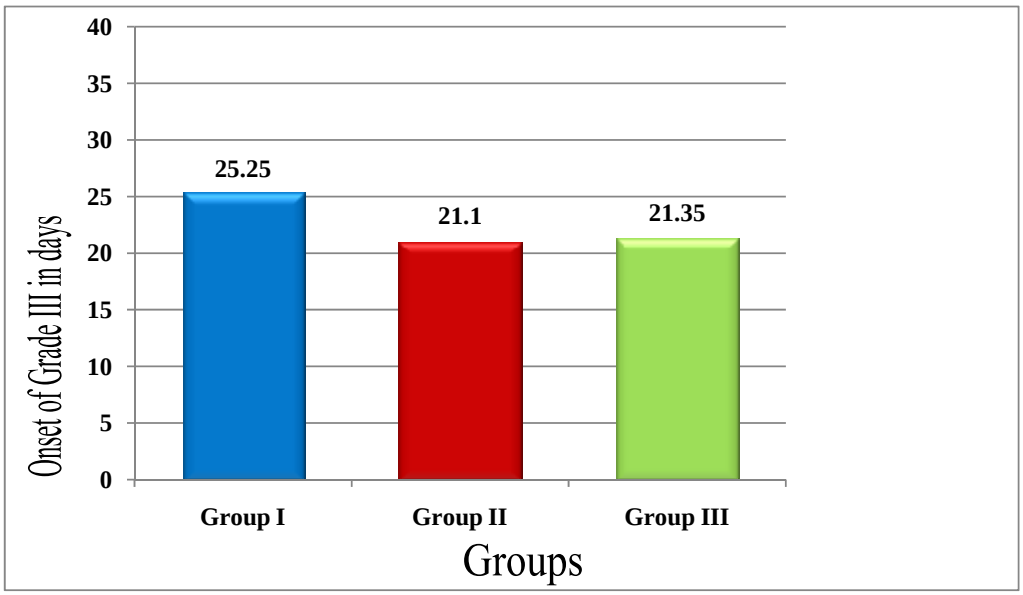

Chart 7 : ONSET OF GRADES III

Grade IV

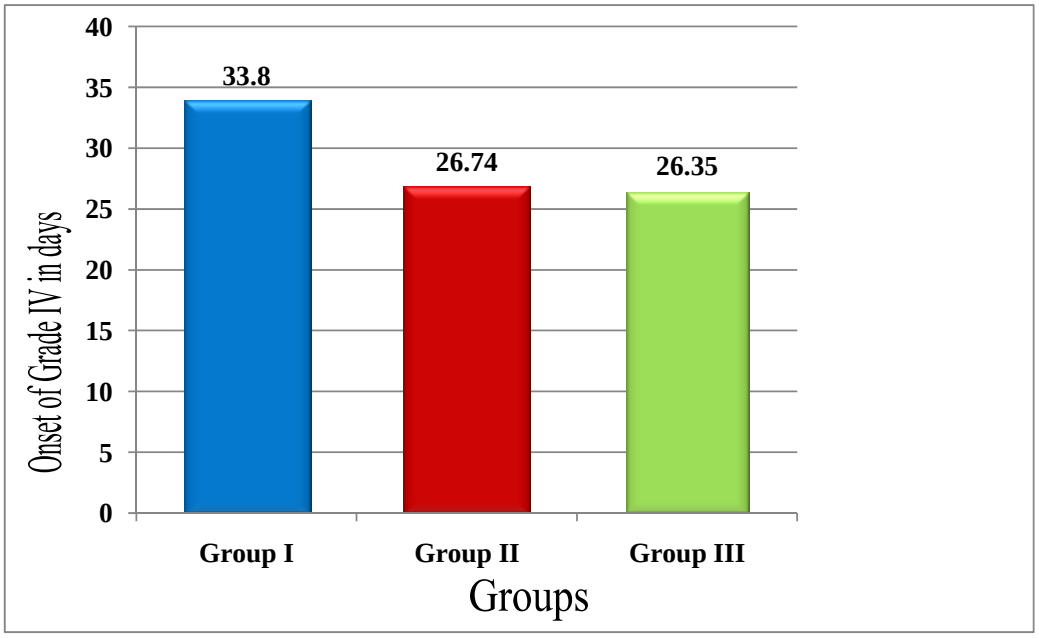

Chart 8 : ONSET OF GRADES IV

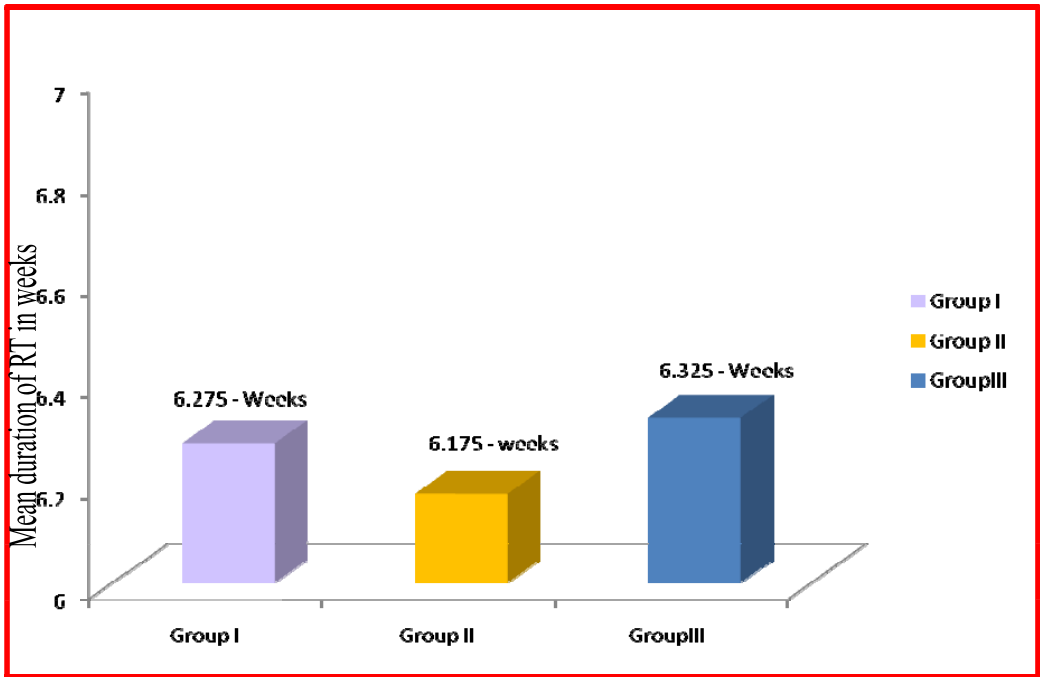

Chart 13 : Duration Of Radiotherapy

Group 1

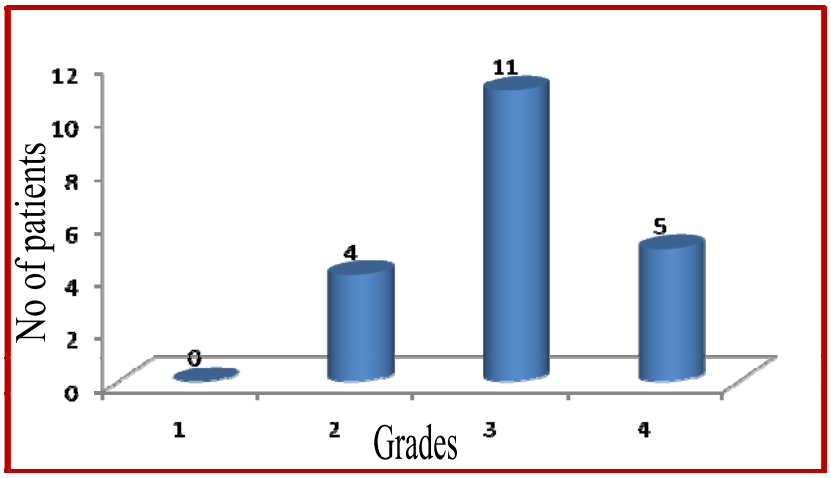

Chart 14

Group 2

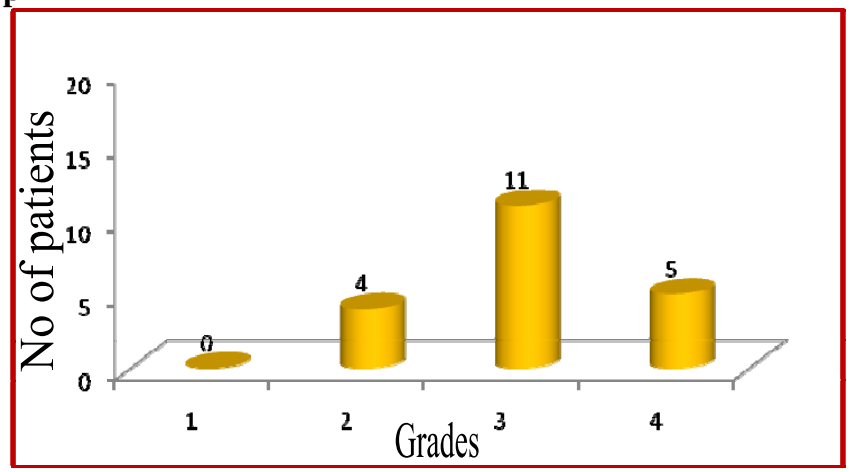

Chart 15

Group 3

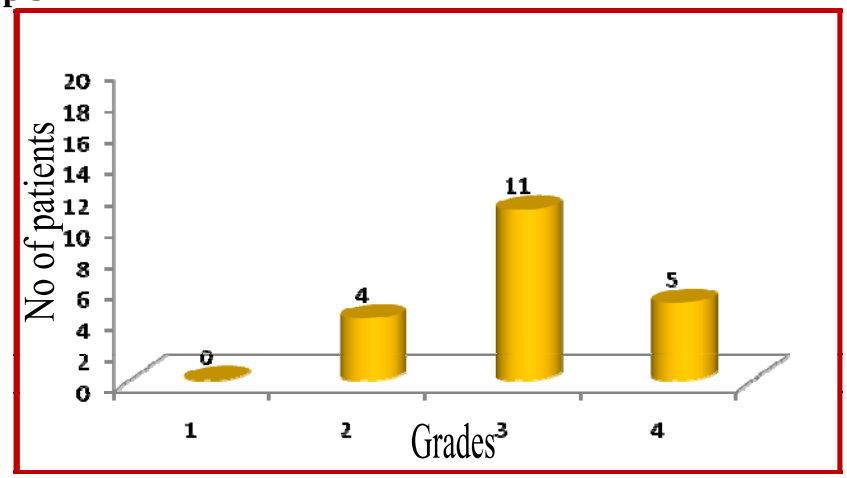

Chart 16

Post RT End Of First Week

Group 1

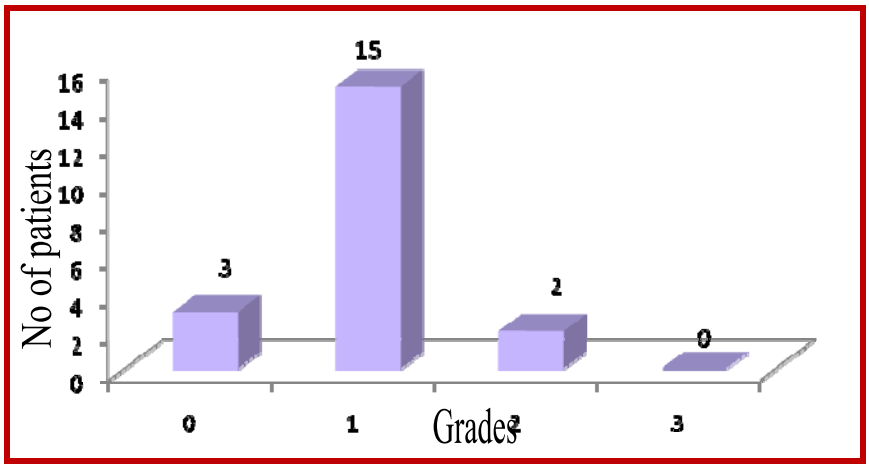

Chart 17

Group 2

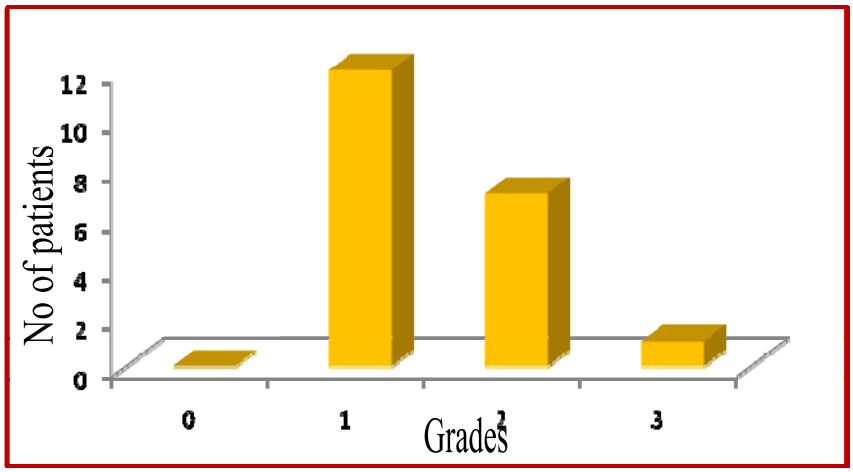

Chart 18

Group 3

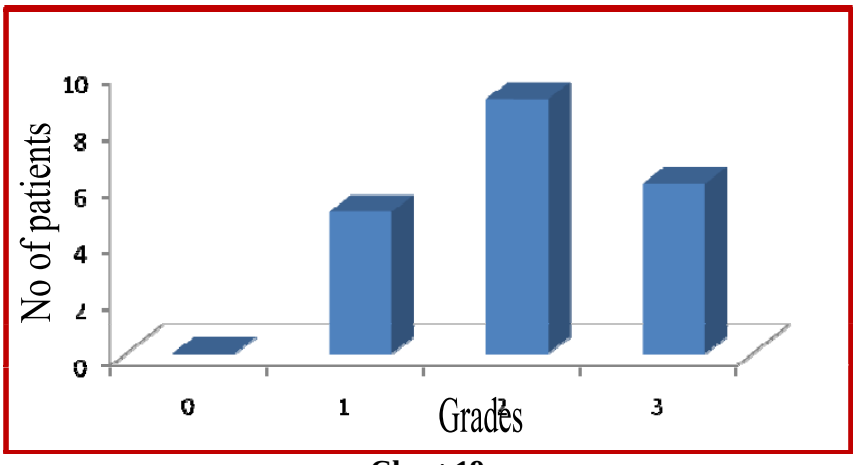

Chart 19

Post Rt End Of Second Week

Grade 1

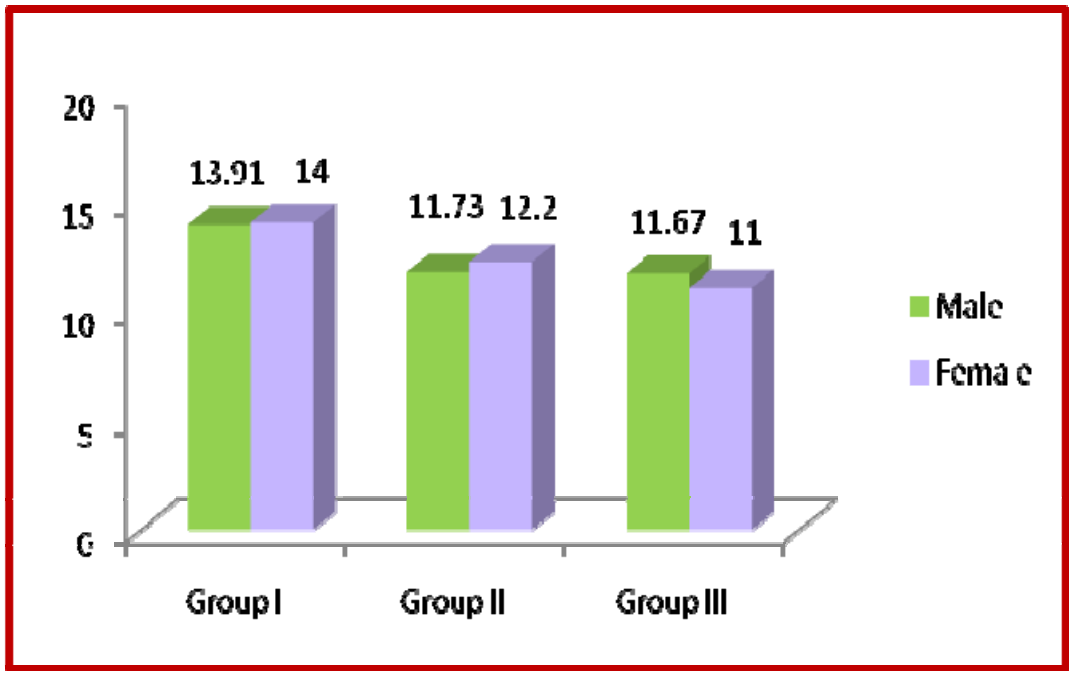

Chart 9

Grade 2

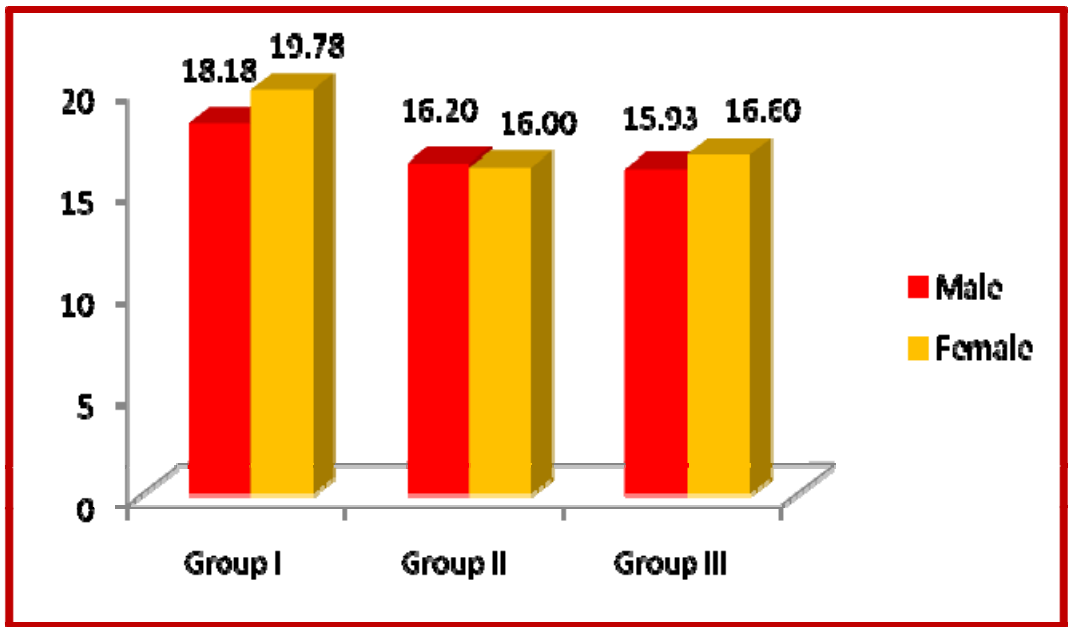

Chart 10
Minimum, Maximum and Mean days of onset of Grades I, II, III, & IV in Groups

Grade 3

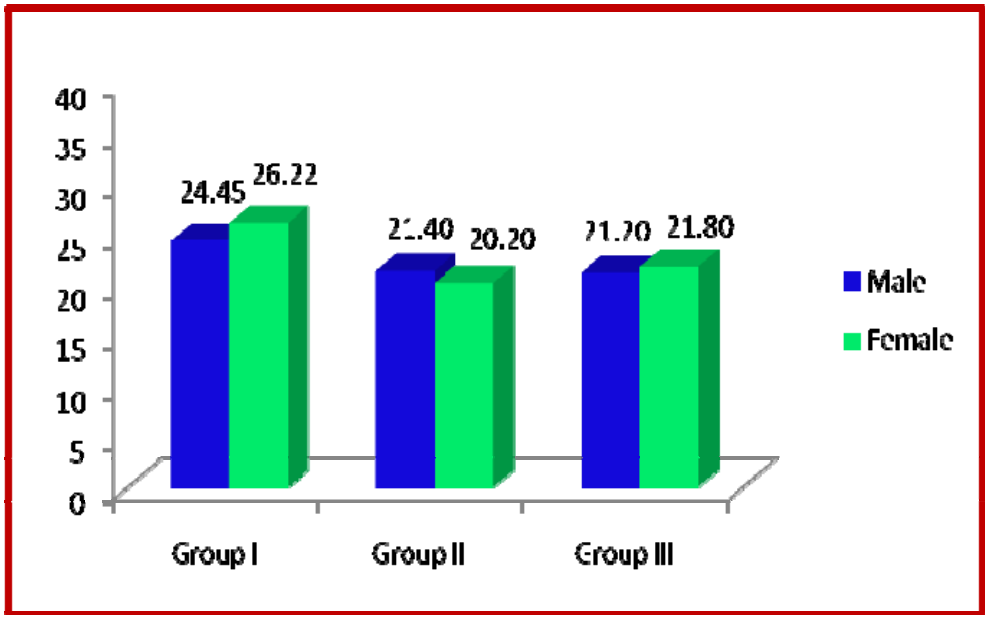

Chart 11

Grade 4

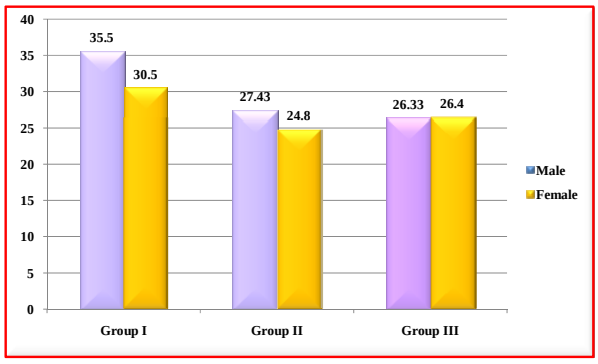

Chart 12

Minimum, Maximum and Mean days of onset of Grades I, II, III, & IV in Groups

DISCUSSION

A large number of malignancies of oral and oro-pharyngeal region are radio-sensitive squamous cell carcinomas and hence irradiation is used as a primary treatment modality, for the management of these malignancies. There is always some degree of damage to normal tissues as a consequence of the therapeutic regimen (which may go as high as 60-70 Gy)

Oral complications arise from radiation injury to the Oral mucosa and tongue, salivary glands, oral musculature and alveolar bone. Radiation-induced mucositis is a normal accompaniment of radical radiotherapy to the head and neck region. Normally, the oral mucosa has a relatively high cell turnover rate. Exposure to ionizing radiation leads to mucosal erythema, small whitish patches[6] and ultimately results in confluent mucositis. In the later phases, oral ulceration and bleeding become a dose-limiting toxicity. Mucositis is a result of imbalance between cell loss and cell proliferation. The intensity of mucositis can be altered by new fractionation schedules, concurrent chemo-radiotherapy and co-morbid medical conditions. Bacterial colonization in the oralmucosa can aggravate the preexisting mucositis. Endotoxins released from the gram-negative bacilli are potent mediators of the inflammatory process in the oral mucosa. oropharyngeal flora

too contributes to the radiation-induced mucositis[9,41] other factor like poor oral hygiene and tobacco chewing and smoking habits can contribute to radiation- induced mucusitis**.** Various agents have been tried for management of radiation-induced mucositis. The agents recommended or tested for the prevention and management of radiation induced mucositis, have targeted specific pathways and they include mucosal coating agents, anti-inflammatory agents, antimicrobials, immunomodulators, anesthetics and analgesics, and other agents that are difficult to classify. These locally applied as well as systemically taken agents have been supportive at most, consisting of measures to alleviate pain and improve discomfort, support adequate hydration and, in some, the ability to eliminate secondary infections[89]**.**

In 1981,WHO published grading criteria for 28 acute toxicities, including mucositis[41]**.**Subsequently,the National Cancer Institute's(NCI) common toxicity criteria was published in 1983,which included 49 chemotherapy related toxicity criteria scales along with mucositis. [85]

In the present study, 60 patients were selected for the study. There were 41 (68.3%) males and 19 (31.7%) females. The minimum age of male was 34 years and the maximum age was 70 years. The minimum age of female was 30 years and the

maximum age was 65 years. These patients were planned for radiotherapy. Cobalt- 60 equipment was used for radiation treatment.

Conventional fractionated radiation was delivered to the tumour volume at a dose rate of 2 Gy per fraction, treating five fractions per week to a total period of 6-7 weeks. The weekly dosage was 10 Gy(Gray).The total dosage given varied from 60 to 70 Gy(6000 to 7000 cGy)

Radiation source, modality, field size, treatment areas, total planned dose, number of fractions, days of irradiation were recorded in the structured proforma prepared for the study. The 60 patients were randomly divided into two groups. Group 1(n=20): twenty patients in Group 1 were instructed to rinse with 20 ml of pure natural honey,15 minutes before,15 minutes after and 6 hours post-RT, during the course of radiotherapy.

Group 2(n=20): twenty patients in Group 2 were instructed to rinse with 15 ml of 0.15%w/v benzydamine hydrochloride ,15 minutes before,15 minutes after and 6 hours post-RT, during the course of radiotherapy.

Group 3(n=20): twenty patients in Group 3 were instructed to rinse with 15 ml of 0.9% normal saline,15 minutes

before,15 minutes after and 6 hours post-RT, during the course of radiotherapy.

During the course of radiotherapy, two patients in the group 3 were found to be having oral candidiasis, during the period of study. They were advised good oral hygiene with topical 1% Clotrimazole mouth paint.

The onset of Grade I (WHO Mucositis Grading) was clinically observed in all the 3 groups, and severity is clinically observed by the days of onset of grades II,III,& IV in the 3 groups and also the number of patients with more severe clinical and functional grades of III & IV.

The present study revealed that patients in Group 1(Pure natural honey group) had a delayed onset of mucositis, compared to Group 2 (0.15% w/v benzydamine Hcl group) and Group3 (0.9% normal saline group) (14^{th} day in group1 compared to 12^{th} day in group 2 and group. The p-value between group1 & 3 is 0.001, between group2&3 is 0.679, and between 1&2 was <0.001.

The group1 had a statistically significant difference on the onset, compared to the groups 2 and 3. The present study revealed that patients in Group 1(Pure natural honey group) had a late onset of Grade II mucositis, compared to Group 2(0.15% benzydamine Hcl group) and Group3 (0.9% normal saline

group)(19th day in group1 compared to 16th day in group 2 and 16th day in group3. The p-value between group 1 & 3 is < 0.001, between group 2 & 3 is 0.997, and between 1 &2 was <0.001.

The group1 had a statistically significant difference on the onset of grade II, compared to the groups 2 and 3. The present study revealed that patients in Group 1(Pure natural honey group) had a late onset of Grade III mucositis, compared to Group 2(0.15% benzydamine Hcl group) and Group3(0.9% normal saline group)(25th day in group1compared to 21st day in group 2 and 21st day in group3. The p-value between group1 & 3 is <0.001, between group2&3 is 0.965,and between 1&2 was <0.001.

The group1 had a statistically significant difference on the onset of grade III, compared to the groups 2 and 3. The present study revealed the out of 20 patients in group 1, only 6 (30%) patients developed more debilitating grade IV mucositis, compared to group 2 whereby 19 patients out of the 20 patients developed grade IV mucositis and group 3 Whereby all the 20 patients developed grade IV mucositis. This observation is consistent with the study by B.M.Biswal, A.Zakaria, N.M.Ahmad in 2002[6], wherein they reported that there is significant reduction in symptomatic grade III and IV mucositis

in honey treated group. In the same study, the mean onset of mucositis both in study and control groups was 3 weeks (No significant difference on the onset of mucositis). [4] In this study, the mean onset of mucositis in honey treated patients(Group 1) were on 14th day, compared to 12th day in 0.15% benzydamine treated (Group 2) patients and 12th day. The difference between Group 1 and the groups 2&3 was statistically significant (p=<0.001).

The observation of 14 (70%) patients of the total 20 patients in group 1 ,to have not developed grade IV mucositis, in our study, also is consistent with the study by Rashad U M et al in 2006 ,wherein no patients in the study arm (honey treated) developed grade IV mucositis[63].

This observation is also consistent with study by B.Khanal, M.Baliga,N.Uppal in 2009,wherein it was observed that the proportion of patients with intolerable oral mucositis was lower in honey group compared with controls(p=0.000)[32].

M.Motallebnejad et al in 2004 observed that, in the honey treated group, the mucositis score at the end of each week was significantly lower than the control group.[43]

In this study, we analysed the grades of mucositis everyday, the day of onset of mucositis (grade 1 of WHO mucositis grading) and the days of onset of grade II, III and IV.

The present study also revealed that at the end of post radiotherapy 1st week there was low occurrence of GradeIII in the honey group compared to 0.15% benzydamine and 0.9% saline groups.

Honey forms primarily from the transformation and concentration of nectars from flowers by two processes: The interaction with the upper digestive tract secretion of honeybees and concentration by water loss(>80%) in beehives. They contain moisture, fructose, glucose, sucrose, maltose and other compounds, along With trace elements.[60]

In the recent past, honey has been used for the treatment of burn wound, infected surgical wounds, childhood diarrhea, eye infections, etc[56]

The ancient Egyptians and Greeks used honey for wound care, and a broad spectrum of wounds are treated all over the world with natural unprocessed honey from different sources.[56]

The philosophy of using honey in radiation mucositis was derived from the basic research and clinical observation of rapid epithelization in tissue injuries. [8, 57]

Coating a wound with honey retards tissue oxygenation by sealing the damaged mucosa from air (oxygen).this could dampen pain within 30 seconds after application[56].

Important factors that influence the effectiveness of honey are (1) Its hygroscopic nature,(2) Acidic pH prevents bacteria growth when applied to the mucosa;(3) Inhibin (hydrogen peroxide) converted from glucose oxydase and gluconic acid;(4)Enzymes (growth factors) and tissue-nutitive minerals and vitamins help repair tissue directly. The reduction of radiation-induced mucositis in honey-treated patients might be due to the bacteriostatic effect of viscid honey. Pure honey is acidic, with a ph of around 3.9.The solubility reducing factor present in honey can activate in absence of saliva. Honey applied on radition-induced xerotic mucosa increases the micro-hardness of enamel, thereby preventing caries. Hence, it has been postulated that honey is less cariogenic in dry mouth patients[83].

In a report of the Russian academy of Medical Science, patients treated with honey laminolact in uterine cancer patients undergoing radiotherapy showed significant decrease in the severity of radiation-induced intestinal morbidity[84].

Pure honey is ubiquitous, cheap and natural, and exhibits antibacterial, analgesic and tissue nutritive factors to stimulate re-epithelialisation in the damaged mucosa, and is thereby a justified agent to try in radiation mucositis.

SUMMARY AND CONCLUSION

In the present study, 60 patients were selected for the study. There were 41 (68.3%) males and 19 (31.7%) females. The minimum age of male was 34 years and the maximum age was 70 years. The minimum age of female was 30 years and the maximum age was 65 years. These patients were planned for radiotherapy. Cobalt- 60 equipment was used for radiation treatment. Radiation source, modality, field size, treatment areas, total planned dose, number of fractions, days of irradiation was recorded in the structured proforma prepared for the study. The 60 patients were selected for the study all the patients were given habit counseling regarding stopping of the habit of the tobacco and were advised on basic oral hygiene. These 60 patients were randomly divided into three groups.

Twenty patients in Group 1 were instructed to rinse with 20 ml of pure natural honey,15 minutes before,15 minutes after and 6 hours post-RT, during the course of radiotherapy. Twenty patients in Group 2 were instructed to rinse with 15 ml of 0.15%w/v benzydamine hydrochloride ,15 minutes before,15 minutes after and 6 hours post-RT, during the course of radiotherapy. Twenty patients in Group 3 were instructed to rinse with 15 ml of 0.9% normal saline, 15 minutes before,15

minutes after and 6 hours post-RT, during the course of radiotherapy.

The onset of Grade I (WHO Mucositis Grading) was clinically observed in all the 3 groups, and severity is clinically observed by the days of onset of grades II,III,& IV in the 3 groups and also the number of patients with more severe clinical and functional grades of III & IV.

The onset of grades I,II,III&IV were compared within groups. The grades during end of post-RT 1^{st} week and 2^{nd} week were compared between groups.

Statistical analysis were done using SPSS version 15 days of onset of grades I, II, III & IV were compared between groups 1, 2 & 3. From the presence study, we found usefulness of pure natural honey in the management of symptomatic radiation mucositis. As this agent is effective in radiation mucositis, the same treatment could be useful in the management of chemotherapy-induced oral stomatitis/mucositis and in mucositis of bone marrow transplant patients. The philosophy of management in the above conditions is similar. The further issue in the use of medicinal honey is quality assurance of natural honey-which might be different in different geographic locations-and the source of pollens.

As the future multi-modality approach to cancer lies in chemo-radiotherapy and altered fractionation schemes, prevention of oral mucositis is very important in its management. Honey could be a simple, potent and inexpensive agent, which is easily available, especially in the present Indian scenario for the management of this morbidity. However, further randomized studies are essential to validate our findings.

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